# HILGARDIA

A JOURNAL OF AGRICULTURAL SCIENCE

PUBLISHED BY THE

CALIFORNIA AGRICULTURAL EXPERIMENT STATION

VOL. 4 MARCH, 1930 NO. 12


# VACCINATION OF CALVES AGAINST TUBERCULOSIS WITH CALMETTE-GUÉRIN CULTURE, BCG


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## INTRODUCTION

When in June, 1924, Calmette and Guérin[20] first published their offer to furnish BCG cultures for experimental trial, the writers took under consideration plans for testing this method of vaccinating animals against tuberculosis. The matter rested until 1926, however, when Mr. H. D. Williamson of Napa, having read press notices of the French experiments, became interested in the possibility of controlling tuberculosis by means of this material in his dairy herd of about two hundred cows on Grisly Island in the Sacramento-San Joaquin Delta. Mr. Williamson urged that cultures be secured at once and furnished funds to pay for cablegrams and airmail postage. Plans were then developed for conducting formal investigations under controlled conditions at the California Agricultural Experiment Station, as well as for starting the work with the culture in the Williamson herd.

The following paper includes a summary of the experiments with the culture BCG by Calmette and Guérin and others, a report of the investigations with the culture to date at the California station, and an account of the attempt to control tuberculosis by means of culture BCG in a commercial dairy herd, together with summaries and conclusions drawn from the work in California.


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## SUMMARY OF THE EXPERIMENTS OF CALMETTE AND GUÉRIN AND OTHERS

The experiments by Calmette and Guérin concerning the resistance to tuberculosis conferred upon young cattle by the bile-attenuated tuberculosis culture BCG (bacille bilié Calmette-Guérin) may be summarized as follows:

In 1911 Calmette and Guérin[15] published the results of experiments in which 14 heifers, about 3 years of age, were given each month intravenous doses of 200 milligrams of BCG. After 6 months of such treatment, 9 became cachectic, but, on slaughter, no lesions of tuberculosis were found. Attempts were made to infect some of the remaining vaccinated heifers by injecting cultures of virulent bovine tubercle bacilli intravenously. It was determined by guinea-pig tests that these heifers excreted the virulent bacilli by way of the intestinal tract, but when they were slaughtered, no visible lesions of tuberculosis could be found.

In 1913 and 1914 Calmette and Guérin[17, 18] reported that 7 heifers had been vaccinated intravenously with two doses of 1 and 5 mg, respectively, of the thirty-third and thirty-fourth transplants of BCG on ox-bile potato. Thirty days after the second vaccination, a dose of 3 mg of virulent tubercle bacilli was given intravenously to each of the 7 heifers and to 1 nonvaccinated control. The control died of miliary tuberculosis in 34 days. The vaccinated heifers remained apparently normal and were killed at 1, 2, 3, 4, 8, 12, and 18 months, respectively, after the test injection. No visible lesions of tuberculosis were found, but in all 7 cases virulent tubercle bacilli were demonstrated by guinea-pig inoculation in the apparently normal bronchial lymph nodes.

In 1920, Calmette and Guérin[19] published the results of a carefully controlled trial, begun in 1912, of the resistance to tuberculosis of vaccinated heifers when associated with cows having open tuberculous lesions. Ten non-reacting heifers, aged 9 to 10 months, were selected, 4 of them being reserved as controls. Each of the other 6 received, in the jugular vein, 20 mg of bacilli from a 2-week-old planting of BCG, the seventieth transplant on ox-bile potato. On the same day, the 10 heifers were placed, together with 5 adult tuberculous cows, in a stable arranged to favor natural infection. The experiment lasted for 34 months, during which time some of the 5 tuberculous cows died and were immediately replaced by other open cases of

tuberculosis. After 18 months of such exposure to tuberculosis, none of the 6 vaccinated heifers reacted to tuberculin, while 3 of the controls gave definite reactions.

Three of the 6 vaccinated heifers, at the end of a year, received intravenously a second injection of 20 mg from a 21-day culture of BCG, the eighty-ninth transplant on ox-bile potato, and 2 of these 3 heifers received again, 12 months later, 20 mg of the one hundred and thirteenth generation of BCG culture. In March, 1915, 1 of these 2 heifers was killed accidentally. No lesions of tuberculosis could be found, and guinea pigs inoculated with portions of her bronchial lymph nodes were found normal on autopsy after 5 months.

On account of the war, it became necessary to terminate the experiment in August, 1915. On autopsy of the 9 remaining heifers, the following observations were made:

1. Three controls presented some tuberculous lesions, which were in general of slight extent. The fourth control, which had never reacted to tuberculin, was free from lesions.

2. Two of the 3 heifers vaccinated only in 1912 had a few small tubercles in the lungs and in the bronchial, mediastinal, and mesenteric lymph nodes. The third was free and portions of its lymphatic tissue inoculated into guinea pigs did not cause tuberculosis.

3. The heifer which had been vaccinated in 1912 and again in 1913 was free from tuberculous lesions, and portions of its lymphatics, injected into guinea pigs, did not cause tuberculosis.

4. The 2 heifers vaccinated in 1912, 1913, and 1914 were free, and portions of their lymphatics failed to produce tuberculosis in guinea pigs.

Calmette and Guérin concluded from this experiment that the intravenous injection of BCG not only confers upon healthy bovines a tolerance against artificial test inoculation, but also protects them for a time against infection from close and continued cohabitation in infected stables. They concluded that this tolerance, apparently linked with the presence of nonvirulent bacilli in the body, does not last longer than 18 months after a single vaccination. They stated that the tolerance could be maintained by vaccinations carried on each year.

In 1924, Calmette and Guérin[20] reported an experiment with 20 heifers, 7 to 8 months old and free from tuberculosis as indicated by the tuberculin test. Heifers 1 to 12 were vaccinated subcutaneously in the dewlap with BCG, the even numbers receiving 50 and the odd numbers 100 mg, respectively. Numbers 13 to 18 served as controls and were maintained under the same conditions. The 12 vaccinated heifers were divided into six groups of 2 animals each (1–2, 3–4, etc.).

With each group was placed one of the nonvaccinated control heifers, each group of three occupying an isolated stall. To test the resistance conferred by the vaccine, virulent bovine tubercle bacilli from cultures were weighed in a fresh state and inoculated into 19[5] of the heifers, intravenously, in 5-mg doses with the following results:

Group I. Vaccinated heifers 1 and 2 and control No. 13 were inoculated 1 month after vaccination. Control No. 13, killed 60 days after inoculation, showed advanced miliary tuberculosis of the lungs. Vaccinated heifer No. 2 died of verminous bronchitis 5 months and 7 days after inoculation. No lesions resembling tuberculosis were found. Guinea pigs, inoculated with portions of the apparently normal left bronchial lymph node, contracted tuberculosis. Vaccinated heifer No. 1 remained in good condition and was butchered 12 months after inoculation. No lesions of tuberculosis could be found. Portions of the left bronchial lymph node were injected into 4 guinea pigs. These were killed but no indication of tuberculosis could be found in them.

Group II. Vaccinated heifers 3 and 4 and control No. 14 were inoculated 3 months after vaccination. Control No. 14 died of miliary tuberculosis 44 days after inoculation. Vaccinated heifers 3 and 4 remained in good condition. They were slaughtered 11 months after the test inoculation and no tuberculous lesions could be found. Portions of their bronchial lymph nodes were inoculated into guinea pigs. These pigs were killed 45 days after inoculation. Those that had received tissue from heifer No. 4 were tuberculous, but those inoculated from No. 3 were free from tuberculosis.

Calmette[12] in the latest edition of his book *L'Infection Bacillaire et la tuberculose chez l'Homme et chez les Animaux* attributes great importance to the observation that the guinea pigs inoculated from heifers 1 and 3 remained free from tuberculosis. In previous experiments he had observed that calves vaccinated by the intravenous route and later inoculated by the same route with virulent bacilli, remained free from caseous lesions, but their apparently normal lymph-node tissue would retain the bacilli virulent for guinea pigs for at least 18 months. The fact that guinea pigs did not contract tuberculosis when injected with tissues from heifers 1 and 3 was interpreted by Calmette to indicate that the subcutaneous injection of BCG is more efficacious than the intravenous method of vaccination.

Group III. Vaccinated heifers Nos. 5 and 6 and control No. 15 were inoculated 6 months after vaccination. Control No. 15 became very emaciated, was killed 60 days after inoculation and was found to

[5] Only 19 heifers are accounted for in their descriptions.

have extensive miliary tuberculosis of the lungs with some hepatization. Vaccinated heifers 5 and 6 were slaughtered 8 months after inoculation and no lesions of tuberculosis were found, but their apparently normal bronchial lymph nodes contained tubercle bacilli virulent for guinea pigs.

Group IV. Vaccinated heifers 7 and 8 and controls 16 and 16*A* were inoculated 12 months after vaccination. On account of the long delay, it was thought necessary to have 2 controls. The 2 controls died of miliary tuberculosis in 32 and 58 days, respectively. The vaccinated heifers Nos. 7 and 8, remained in excellent condition and were slaughtered 3 months after the infecting inoculation. No tuberculous lesions could be found, but their apparently normal bronchial lymph nodes contained tubercle bacilli virulent for guinea pigs.

Group V. Vaccinated heifer No. 10 died 25 days after vaccination. Vaccinated heifer No. 9 and control No. 17 were inoculated with virulent bacilli 15 months after vaccination. Control No. 17 died of miliary tuberculosis 54 days after inoculation. Vaccinated heifer No. 9 was butchered 2 months after inoculation. No lesions typical of tuberculosis were found, although the bronchial and mediastinal lymph nodes were reported by the veterinary director of the abattoir to be slightly enlarged and juicy. No mention was made of guinea-pig inoculations.

Group VI. Vaccinated heifers 11 and 12 and control No. 18 were inoculated 18 months after vaccination. The control died of miliary tuberculosis in 42 days. After the intravenous inoculation, with virulent tubercle bacilli, No. 11 gave the immediate thermal reaction typical in vaccinated calves, while No. 12 did not show this reaction but, on the 13th to the 18th days it showed a marked hyperthermia (39.8° C), after which it became apparently normal in every way. The 2 vaccinated heifers were killed 2 months after inoculation. Vaccinated heifer No. 11 was apparently free from tuberculosis, although its lymph nodes were somewhat enlarged and soft. In vaccinated heifer No. 12, the lungs were firm and incompletely collapsed. On section, the lung tissues were found thick and hard, but permeable throughout. The bronchial and mediastinal lymph nodes were firm and enlarged to twice normal size. On section, they were found to contain white islets, inclining to gray, surrounded by a zone mixed with blood, almost hemorrhagic, without visible tuberculous nodules. Calmette concluded that No. 12 had reached the limit of its tolerance after 18 months, although its resistance was still sufficient to prevent it from contracting the form of acute miliary tuberculosis shown by the control.

In 1927, Guérin, Richard, and Boissiere[37] reported an attempt, begun in 1921, to eliminate tuberculosis from an infected herd of 50 to 70 cattle at Gruville by vaccination of the young calves with BCG. The harmlessness of BCG for cattle was indicated by the fact that 30 animals, vaccinated in 1921–1924 and in several cases revaccinated for 3 or 4 consecutive years, were found, on slaughter for beef, to be free from tuberculous lesions. Guérin and his associates expressed a belief in the efficacy of the vaccine in preventing tuberculous infection in the young cattle associated with many tuberculous cows under conditions in which no sanitary precautions were taken to prevent the spread of infection. However, definite proof of this seems to be lacking, especially since no controls were used. No precautions appear to have been taken to protect the young calves from infection during the first few weeks of their lives and, in several instances, the first vaccination was delayed from 3 to 8 weeks, although in a recent publication, Calmette and Guérin[21] have pointed out the importance of protecting all vaccinated calves in herds where cases of open tuberculosis exist by observance of the following precautions:

(1) The first injection of BCG vaccine should be made as soon as possible after birth and not later than the 15th day.

(2) The vaccinated subjects should be protected from abundant and repeated contamination by isolation and by feeding milk free from tubercle bacilli.

They further state that if calves receive the harmless bacillus-vaccine before exposure to virulent bacilli, they will be "premune."[6] If, on the contrary, virulent organisms are already installed in the animal the vaccine cannot play any useful rôle.

Attempts to confirm the findings of Calmette and his associates have been reported by investigators in various countries. So far as the experiments on cattle are concerned, the results have been variable.

Sanz[64] has reported the vaccination of 841 head of cattle of all ages, 286 of which were in herds from which reactors to the tuberculin test were removed before starting to vaccinate. No injurious effect was observed to result from the vaccine.

Fénelon[33] has reported preliminary results of a field trial on two infected farms where all of the calves have been vaccinated since 1924 at 3 weeks to 1 month of age. One hundred and eleven vaccinated calves on these farms, at the time of publication, were reported to be

[6] According to Calmette, the word "premunition" was first proposed in 1924 by Edm. Sergent and Donatien to designate a condition of protective latent infection, such as exists in certain protozoan diseases, particularly bovine piroplasmosis.

in excellent condition. No tuberculous lesions were found in 32 vaccinated calves that were butchered or that had died of intercurrent diseases.

The results of Ascoli and his associates[1] have apparently supported the claim that subcutaneous injections of BCG will protect against an intravenous dose of virulent tubercle bacilli that is fatal to nonvaccinated cattle.

A preliminary report of the Ukranian Commission summarized by Tzeknovitzer[69] states that 12 calves and 2 colts were injected subcutaneously or intravenously with 76 to 200 mg of BCG. They all continued to develop in a normal fashion. One of the calves which had received 76 mg subcutaneously was killed 6 months later. Autopsy showed it to be free from tuberculosis, except that at the point of vaccination there was a circumscribed infiltration of the tissues with acid-fast organisms.

Eight months after vaccination, another calf which received 300 mg intravenously was killed. Lymph nodes in various parts of the body were enlarged, but no lesions characteristic of tuberculosis could be found. Guinea pigs inoculated from the lymph nodes remained normal.

In a communication in 1927 Tzeknovitzer[70] reported a trial with 13 vaccinated and 4 control calves. Six to 8 months after vaccination these were subjected to intravenous infection with the small dose of 0.005 mg of Vallée's strain of virulent tubercle bacilli. His statement of the results may be summarized as follows:

After the injection of the virulent Vallée culture the controls did not show a thermic reaction, but the vaccinated animals had a typical rise in temperature corresponding to a tuberculin reaction. All of the controls died in about 6 weeks of miliary tuberculosis. One of the vaccinated animals died 6 weeks after infection. No evidence of tuberculosis was found in it, but there was impaction of the stomachs and fatty degeneration of the liver. The other vaccinated animals developed normally. Two were butchered 3 months after the intravenous infection. Autopsy showed a nodular tuberculosis of moderate intensity in one animal and a similar, but milder, condition in the other. These lesions were not sufficient to have any clinical effect on the general condition of the animals. One of the vaccinated calves died from causes other than tuberculosis 3½ months after intravenous infection. In the left lung were 7 to 10 small isolated tubercles tending to calcification. Two more of the vaccinated calves were butchered 6 months after infection. One had several isolated tubercles on the pleura and a large number scattered through the parenchyma of the

lung, but it was stated that there was no evidence of a tendency of these lesions to spread. The other calf, killed at the same time, had several calcified nodules in the bronchial and mediastinal lymph nodes and also a few pulmonary nodules. There was a slight hyperplasia of the pleura.

Another vaccinated calf was butchered 8 months after infection and showed 3 tubercles in the lungs and 1 on the pericardium. There was a slight serous hyperplasia of the pleura.

Ten months after vaccination, another vaccinated calf was butchered. In the lower part of the lung was a caseous mass, the size of a hazel nut and 6 or 7 others, each the size of a pea. One tubercle was found in the apex of the left lung. The nature of the caseous foci had not been determined at the time of publication. There was a slight hyperplasia of the visceral and parietal pleura.

All of these cattle killed 6, 8, and 10 months after infection, were reported to be clinically in excellent general condition.

In the third report of the Ukranian Commission, Tzeknovitzer[71, 72] stated that 50 calves in all had been used in the experiments and that the vaccination of cattle confers in general in these animals a manifest resistance to experimental infection.

Lange and Lydtin[44, 45] vaccinated calves in various ways and 3 to 3½ months later infected them with intravenous doses of 2 mg of the Vallée strain of virulent bovine tubercle bacilli with the results shown in table 1.

In a further vaccination trial, Lange and Lydtin[45] injected 4 calves intravenously with 20 mg of Vallée strain of virulent bovine tubercle bacilli, 6½ months after vaccination of 2 of them. The results of this trial are shown in table 2.

They concluded that their results stand in contradiction to the observations of Calmette and Tzeknovitzer, and suggested that, in recent years, there may have been a weakening in virulence of BCG, thereby resulting in a reduction in its immunizing power.

Watson, McIntosh, and Konst[77] have reported experiments with 14 calves that were placed with tuberculous cattle within the week following vaccination. On slaughter after 15 to 25 months these calves all showed tuberculous lesions, varying in the different animals from involvement of two groups of lymph nodes to extensive generalization. The control animals, 3 in number, also showed lesions. Their conclusion was that as far as a comparison is possible with these unequal numbers, there was nothing in favor of the vaccinated animals, nor any evidence to indicate a greater degree of resistance on their part to tuberculosis.

In addition, Watson[76] has reported results on 7 calves which were vaccinated twice at intervals of 12 to 14 months. These and 3 unvaccinated controls were all exposed to natural infection through milk and by cohabitation with tuberculous cattle. On slaughter at an age of 19 to 25 months tuberculous lesions were found in all 10. Watson stated that there was no definite evidence that the vaccinated were more resistant than the unvaccinated.

TABLE 1

RESULTS OF VARIOUS METHODS OF VACCINATION IN TESTS BY LANGE AND LYDTIN*

| Calf No. | Vaccination | Death, days after infection | Autopsy |
|---|---|---|---|
| 2 | 160 mg BCG subcutaneous | Died, 56 days | Miliary tuberculosis of lungs. Closely studded with miliary to linseed-sized nodules. Bronchial lymph nodes enlarged without macroscopically visible nodules. Very fatty liver. No other changes of significance. |
| 5 | 160 mg BCG subcutaneous | Killed, 75 days | Lungs flecked with hardly visible spots. Microscopical accumulations of round and spindle cells, typical of tubercles. Bronchial nodes had no lesions. Kidneys had many typical tubercles, microscopic in size. |
| 7 | 160 mg BCG subcutaneous | Killed, 75 days | Lung flecked with discrete linseed-sized nodules. Microscopically like No. 5. No other lesions of significance. |
| 21 | 160 mg BCG subcutaneous | Killed, 78 days | In apical lobes of lungs, many linseed-sized tubercles with yellow centers. Many such nodules in bronchial and mediastinal nodes. Microscopic findings typical of tuberculosis. Kidneys, miliary nodules. |
| 12 | 20 mg BCG intravenous | Killed, 74 days | No lesions found. |
| 1 | 20 mg human strain of tubercle bacilli | Killed, 74 days | No lesions found. |
| 20 | 20 mg human strain of tubercle bacilli | Died, 60 days | Lungs, many pea-sized caseous nodules and miliary tubercles. Arthritis of left tarsal joint. Not possible to determine which lesions were the result of the human bacilli and which the result of the bovine. |
| 9 | Control | Died, 10 days | Bronchopneumonia and miliary tuberculosis of lungs in first stages. |
| 11 | Control | Died, 25 days | Massive miliary tuberculosis of the lungs. Bronchial nodes enlarged without tubercles. |
| 19 | Control | Killed, 74 days | Flecking of the lungs as in cases 5 and 7 with linseed-sized nodules, microscopically typical of tubercles. One kidney tubercle. |
| 22 | Control | Killed, 78 days | Lungs, separated miliary nodules, microscopically typical of tuberculosis as in calf 7. |

* Table taken from the article by Lange and Lydtin.[44]

Rankin[63] after experimenting for 4 years and using about 250 calves reports that the vaccine was entirely harmless, although in most cases the calves reacted to the tuberculin test subsequent to vaccination. The calves which were vaccinated with BCG and immediately exposed to infection showed moderately increased resistance to tuberculosis over unvaccinated controls. Calves vaccinated with BCG and subsequently kept under sanitary conditions for a period in order to permit resistance to develop before exposure to infection, showed 80 per cent immunity as compared to 14 per cent for the controls. He reported that the tuberculous lesions found in the vaccinated calves were in general much less pronounced as well as less numerous than those in unvaccinated animals.

TABLE 2

FURTHER RESULTS OBTAINED BY LANGE AND LYDTIN*

| Calf No. | Vaccination | Death, days after infection | Autopsy |
|---|---|---|---|
| 13 | 100 mg BCG subcutaneous | Died, 25 days | Severe miliary tuberculosis of the lungs and distinct kidney tubercles in all calves. |
| 14 | 100 mg BCG subcutaneous | Died, 17 days | |
| 23 | Control | Died, 23 days | |
| 24 | Control | Died, 23 days | |

* Taken from the article by Lange and Lydtin.[45]

King and Park[40] from observations on 46 calves, 45 of which were still living, concluded that 35 had developed tubercles, *at the point of vaccination at least*, as a result of the subcutaneous injection of 50 mg of BCG, since they became allergic within 12 weeks after vaccination, but 27 of 29 had lost skin hypersensitiveness by the 32nd week. In one of these the reaction to tuberculin became doubtful on the 42nd week and was negative at 72 and at 90 weeks. This animal after being transferred to a farm for breeding purposes contracted tuberculosis of the lungs and bronchial and mediastinal nodes.

They have also concluded that 2 out of 8 calves after being fed on BCG showed evidence of protection, the test in one of these animals being the subcutaneous injection of 5.0 mg of bovine tubercle bacilli and in the other of 0.1 mg intravenously. In 5 calves that had been vaccinated subcutaneously and later subjected to the subcutaneous injection of bovine tubercle bacilli, they concluded that there was some evidence of immunity, since in 3 instances the lesion was limited to cold abscess

formation, in 1 there were tuberculous lesions in an adjacent lymph node, and in 1 no lesions were found, whereas in the controls lesions were found in the adjacent nodes in every case except 1, where a cold abscess was present.

In another group of 4 calves vaccinated subcutaneously, 0.1 mg of bovine bacilli intravenously did not produce lesions, while this dose, as well as 0.01 mg, produced lymph-node lesions in 4 control calves. In 2 vaccinated calves 1.0 mg of virulent bacilli produced lesions only in the broncho-mediastinal groups, while the same dose in 2 control calves produced lesions in the mesenteric nodes as well as in the bronchomediastinal. In a feeding infection experiment and also in trials with the subcutaneous injection of 0.01 to 0.10 mg of bovine tubercle bacilli by these writers, the results were inconclusive, since both the vaccinated and control animals failed on autopsy to show any lesions.

Larson and Evans[46] have reported the completion of an experiment begun in 1926 in Illinois with 60 head of cattle under farm conditions. Some of these were vaccinated annually by the subcutaneous injection of 100-mg doses of BCG; others received injections of killed tubercle bacilli suspended in sodium ricinoleate, and a third group were left untreated as controls. Six months after the first vaccination of the experimental animals some tuberculous cattle were turned into a 20-acre field with them. The experimental groups were depleted in various ways, unrelated to the experiment, and the experiment was terminated in 1928, at which time the cattle were slaughtered.

The published autopsy results were in general as follows: Of 7 calves treated with BCG, 4 had tuberculous lesions. Of 8 calves treated with killed bacilli, 2 had tuberculous lesions. Of 6 control calves, 2 had tuberculous lesions. The writers stated that this would seem to indicate that the Calmette method of vaccination had no value whatever in cattle.

Studies at the United States Bureau of Animal Industry Experiment Station have been reported by Schroeder and Crawford,[65] in which vaccinated cattle were exposed, with an equal number of controls, to tubercle bacilli by intravenous and subcutaneous injections, by feeding, and by contact with known tuberculous animals. In the group where exposure was made by feeding virulent tubercle bacilli, they reported more extensive lesions, as a whole, in the vaccinated than in the unvaccinated. In the group infected by injection or natural exposure, including 9 vaccinated cattle and an equal number of controls, it was reported that resistance to the localization and generalization of tuberculosis was slightly more manifest in the vac-

cinated than in the unvaccinated animals, but in no group was there manifest an immunity as measured by the prevention of infection.

A large number of reports have been published on the effect of BCG on small laboratory animals. These have been thoroughly reviewed up to 1928 by Kraus[41] and Lowenstein.[50] An excellent review of the veterinary aspects of the Calmette-Guérin method has been prepared by Zwick and Witte,[80] who claim that infection experiments are of little significance in determining the practical usefulness of an antituberculosis vaccine unless supplemented by trials under commercial farm conditions.

Such a trial has recently been reported by Brinet,[5] who has used the vaccine during the past 6 years on about 300 calves in a dairy near Paris. The records showed that, since 1895, an annual average of 5 or 6 carcass condemnations, partial or total, had been made by inspectors at the time of slaughter of animals marketed from this farm, but that since January, 1926, none have been condemned or even reported to be tuberculous. Among the animals marketed since that date are 35 vaccinated cattle, aged at least 4 years. The owner was quoted as stating that since the vaccine had been used in his herd, the profit and loss sheet had been favorably influenced.

In a recent publication, Gerlach[35] emphasizes the harmlessness of BCG. He reports on the completed experiments and on those still in progress in which 702 guinea pigs and rabbits, 29 monkeys, 381 calves, 46 oxen, 3 dogs, and 2 cats each received 20 to 250 mg of BCG either by the digestive tract or by subcutaneous, intraperitoneal, intravenous, subdural, intracerebral, or intratesticular injection. No matter in what manner the BCG was given, the result was always a localized lesion which gradually healed.

The findings in 10 guinea pigs and 8 rabbits 6 months after 25 mg of BCG were injected intracerebrally led Gerlach to conclude that it is not possible for him to recognize in BCG any infectious character for the central nervous system. In presenting further evidence to support the harmlessness of BCG, he claims that the reinoculation of cultures obtained from inoculated animals failed to augment the virulence and that after successive passages through animals, the virulence is also not increased. He, therefore, considers BCG as a fixed virus.

Gerlach failed to find any increased pathogenicity in the dissociated S variant, as claimed by Petroff,[59] and he is not prepared to recognize in the S colony of Petroff or any other variant a degree of virulence different from the entire BCG culture. These latter conclusions were based on the injection of variants isolated by Gerlach and Gmeiner and also of variants obtained from Tzeknovitzer, Petroff, and from R.

Kraus. Guinea pigs and rabbits and 2 bovines inoculated in these experiments have failed, after 6 months, to show any apparent evidence of disease.

Gerlach further reports on the field vaccination of 371 new-born calves in badly infected tuberculous environments. Thus far, no tuberculous lesions have been found in a number of these animals slaughtered for various reasons, and those still living are reported to be developing normally.

## EXPERIMENTAL METHODS AT THE CALIFORNIA STATION

Great care has been used to follow explicitly the directions given by Calmette[10] in propagating the cultures and preparing the vaccine. The precaution of using separate laboratory equipment and of transferring the stock cultures to bile potato every tenth and eleventh transplant has been observed.

In the controlled experiments at Berkeley and Davis precautions have been taken to protect the calves from virulent infection from birth to vaccination and also during the interval between vaccination and the first intentional virulent infection. At least as many non-vaccinated checks as vaccinated animals have been maintained, and in one of the experiments additional checks treated with heat-killed tubercle bacilli or with nonvirulent strains of acid-fast bacilli were used.

In the experiments on the commercial dairy belonging to Mr. Williamson, only a few controls have been available, and protection of the calves from infection until 30 days after vaccination has not been possible.

*Selection of the Calves.*—The calves described in the artificial-infection experiments at Berkeley and Davis were of the Holstein-Fresian breed, either purebred or the offspring of grade cows and purebred bulls. They were born in the University Dairy Herd at Berkeley or selected from other herds free from tuberculosis as indicated by the tuberculin test. If selected from other tuberculosis-free herds, they were brought to the University Dairy at Berkeley before reaching 10 days of age and reared in an environment believed to be free from virulent bovine tubercle bacilli.

*Use of the Tuberculin Test.*—The question of using the tuberculin test was discussed with Calmette by one of the writers (Traum) in 1926. It was thought desirable that no tuberculin tests should be made on either vaccinated calves or nonvaccinated control calves after they

had been subjected to infection. After 2 years' experimenting without the use of tuberculin on any calves, it was decided to use the test on the nonvaccinated control animals just previous to subjecting them to infection, as an added precaution to insure their being free from tuberculosis. Hence, the control calves subjected to infection since January 1, 1928, have been in all cases tuberculin tested. Also certain calves 4 weeks or older were tested with tuberculin before being vaccinated. Results as shown in tables 7*B* to 11*B* fail to indicate that the use of the intradermic test had any effect on the experimental results.

The test as used at this station consists of a single intradermic injection into a subcaudal fold of 0.1 cc of United States Bureau of Animal Industry intradermic tuberculin, hereafter referred to as B. A. I. tuberculin. This dose is reported by Dorset[7] to be approximately equivalent to 25 mg of O. T.

Continuing the policy of Calmette and Guérin, the International BCG Conference of October, 1928,[27] adopted the ruling: "In order not to modify the receptivity of the animals, the tuberculin test must not be given to any of the experimental animals, either before or during the course of the experiments." The writers at the California station are now of the opinion, however, that it is better experimental procedure to test control calves at the time they are weaned, such a test forming a valuable check on the effectiveness of the methods used to protect all the calves from tuberculous infection during the first 5 or 6 weeks of life. The value of the tuberculin test on calves as a check on the efficiency of the methods used to protect them from infection while being fed on pasteurized milk from tuberculous cows has been demonstrated in the process of eradicating tuberculosis from a dairy of several hundred head of cattle, according to observations by Haring and Traum.[38]

*Preparation of the Vaccine.*—The original cultures of BCG used in the experiments at the California station were obtained by one of the writers (Traum) at the Pasteur Institute, Paris. Four culture tubes of glycerine potato, each labeled *BCG 8* were received by him on April 7, 1926, and were mailed to Berkeley, where they arrived on April 21, 1926. Two additional culture tubes labeled *BCG 1 V-27-26* and *BCG 321 V-30–26* were received on June 16, 1926, at Paris and brought to New York by Traum. These were mailed from New York, arriving in Berkeley on July 10, 1926. Transplants from all except one tube grew vigorously on potato, 5 per cent glycerine broth, Sauton's medium, and 5 per cent glycerine ox-bile potato.

[7] Dorset, M. Personal communication. 1929.

Care has been taken to grow and prepare the vaccine and test it for pathogenicity on guinea pigs in exactly the way prescribed in directions received from Calmette.[10] The stock cultures have been maintained in Roux tubes on potato with 5 per cent glycerine broth for a series of nine generations. The tenth and eleventh generations have been propagated on 5 per cent glycerinated ox-bile potato and then replanted on potato in glycerine broth for nine generations. Old, fully-ripened potatoes are used, since it has been observed that media made from new potatoes give only a feeble growth of BCG. Sauton's medium has been used for propagating some of the vaccine serials. A separate incubator planting room and equipment have been used exclusively for BCG culture and vaccine preparation. The vaccine has been made from cultures not less than 19 days nor more than 26 days old, except in the case of calves vaccinated in April–July, 1926, with material taken directly from the original cultures received from Calmette. When ready for use, each cubic centimeter of vaccine contained 10 mg of BCG bacilli (weight after removal of excess moisture by blotting with filter paper), suspended in a sterile diluent consisting of 100 parts of distilled water, 1 part of chemically pure glucose, and 1 part of chemically pure glycerine, as prescribed by Calmette.[10]

*Method of Vaccination.*—The subcutaneous injections of vaccine into calves have been made by passing an 18-gauge hypodermic needle through the skin of the dewlap in such a way that it passed close to the inner layers of the skin for about an inch. The point of the needle was held firmly against the inner surface of the skin while the 10 cc suspension of BCG was injected to infiltrate the inner layers of the skin as well as the subcutaneous tissue. The purpose of the injection against the skin was to obtain any possible intracutaneous as well as subcutaneous immunizing effect.

*Autopsy Methods and Guinea-Pig Injections.*—All of the cattle at the end of the experiment were slaughtered in small establishments where official inspection was maintained, except those which were showing terminal symptoms of tuberculosis. The latter were killed at a University laboratory and the carcasses taken to a fertilizer works.

The heads, hearts, lungs, livers, and intestines with all the attached lymph nodes were removed in containers to the laboratory. The superficial inguinal or supermammary, the prescapular, and the precrural lymph nodes were removed from the carcass and also taken to the laboratory. The anal, sacral, internal iliacs, sublumbar renal lymph nodes, and those on the stomach were carefully sliced and examined at the abattoir. In this manner all the lymph nodes and

organs with the exception of the brain and some of the deeper unimportant lymph nodes were carefully observed. In the laboratory the lymph nodes were removed and each before inoculation into the guinea pigs was immersed in boiling water from 10 to 12 seconds, placed in sterilized pans or Petri dishes, and sectioned. In a carcass where lesions were very small or entirely absent, the nodes were cut with sterilized scissors into pieces not more than 3 to 4 mm in thickness, several hours being consumed in examination and in obtaining material from each animal for injection. The procedure of immersing lymph nodes in boiling water insured against the introduction of tuberculous infection from the exterior and was far superior to searing with a hot spatula since all crevices and folded tissues could be more readily exposed to the heat, and, besides, any portion of lymph nodes so treated was available for inoculation.

In carcasses where macroscopic evidence of tuberculosis was not present a fair and large composite sample of the lymph nodes and organs was injected into guinea pigs.

The guinea pigs were usually killed between 60 and 90 days after inoculation except in those cases where discharging tuberculous ulcers had developed at the point of inoculation, in which case they were killed at an earlier date.

## THE EFFECTS FROM INTRAVENOUS AND SUBCUTANEOUS INJECTION OF VIRULENT TUBERCLE BACILLI IN VACCINATED AND CONTROL CALVES

In table 3 is summarized the results following the *intravenous infection* of 4 calves that had been vaccinated with BCG and of 4 nonvaccinated controls, and the *subcutaneous infection* of 2 calves that had been vaccinated with BCG and of 2 nonvaccinated controls.

For the infection tests, use was made of the bovine tuberculosis culture 271.[8] The surface growths from 34-day-old transplants were ground for 25 minutes in a mortar and suspended in physiological

[8] The history of bovine tuberculosis culture 271 is as follows: On September 1, 1926, guinea pig 132 was injected intramuscularly with liquid obtained by grinding together tuberculous lesions from 17 cows killed at an abattoir in Berkeley. Guinea pig 132 died of generalized tuberculosis on September 19, 1926, and a portion of its spleen was inoculated into guinea pig 210, which died of generalized tuberculosis on November 5, 1926. The spleen of No. 210 was inoculated into guinea pig 271, which died of generalized tuberculosis on December 22, 1926. Cultures of tubercle bacilli were obtained from the tissues. These proved to be highly virulent for guinea pigs, rabbits, cattle, and swine.

saline solution so that each cubic centimeter contained 0.4 mg of bacilli. From direct microscopic counts it was estimated that each milligram contained about 70 million bacilli.

The 6 animals (Nos. 3, 4, 103, 104, *A* and *B*) listed in tables 3 and 4 that were subjected to infection on January 28, 1929, constitute an experiment planned after reading the statements of Lowenstein[59] and of Petroff, Branch, and Jennings.[60] The former had stated that the proper control for BCG vaccination experiments would be massive injections of killed tubercle bacilli. The latter investigators had claimed that BCG probably had little if any more immunizing power than killed tubercle bacilli. In the same articles, these authors advocated the inauguration of experiments on cattle to confirm or refute Calmette's claims.

The infection trials on calves 1 and 2 at the California station in August, 1927 (table 3) had already supported Calmette's finding that BCG vaccination would protect calves against the rapidly fatal effects of the intravenous introduction of virulent tubercle bacilli, but, on account of Petroff's[58] statements, it seemed desirable to test the effects of intravenous vaccination with BCG and, at the same time, compare it with the effects of a massive intravenous injection of killed tubercle bacilli.

The heat-killed bacilli were prepared from the virulent tuberculosis culture 271 by removing the surface growth from several tubes of Duval's serum egg. The excess moisture was removed by blotting the mass of bacilli between sterile filter paper before it was weighed. Four grams of the damp bacilli were then suspended in 25 cc of Calmette's diluent, placed in a sealed tube and submerged in a water bath at 60° C for 30 minutes. More diluent was then added so that each cubic centimeter contained 5.2 mg of bacilli. This suspension was immediately injected by way of the jugular vein, each calf receiving 192 cc of liquid containing, in all, about 1 gram of killed bacilli. No deleterious effects were observed to follow the injections of killed bacilli. Guinea pigs inoculated intramuscularly with 50-mg doses were found normal on autopsy after 5 months, while guinea pigs injected with one millionth of a milligram before heating developed generalized tuberculosis in a few weeks. In order to eliminate the possibility of confusing lesions produced by the BCG or killed bacilli with those produced by the subsequent intravenous injection of virulent bacilli, 2 calves, Nos. 94 and 96, were retained as noninfected controls (see footnote, table 4).

Extensive trials by the British Royal Commission on Tuberculosis, as reported by Cobbett,[26] indicate that, in calves, infection by the

subcutaneous route affords a very uniform and satisfactory method of testing the virulence of various strains of bovine tubercle bacilli and is also a delicate test of the variations in resistance to tuberculosis in individual animals. At the California station such a test of the variation in resistance between 2 vaccinated and 2 control calves has been carried out, with results as shown for calves 5, 6, 105, and 106 in table 3.

TABLE 3

RESULTS FROM INTRAVENOUS OR SUBCUTANEOUS INFECTION OF VACCINATED AND CONTROL CALVES

| Method of injecting virulent tubercle bacilli | No. | Born | BCG vaccination | | Intervals in days | | | Remarks For detailed autopsies see pp. 325-332 |
|---|---|---|---|---|---|---|---|---|
| | | | Point of injection | Amt. mg. | Birth to vaccination | Vaccination to infection | Infection to autopsy | |
| | | 1927 | | | | | | |
| | 1 | Mar. 22 | Dewlap | 100 | 8 | 140 | 138 | Remained apparently normal until butchered. Apparently normal lymphatics caused tuberculosis in guinea pigs. |
| | 2 | Apr. 18 | Dewlap | 100 | 4 | 117 | 309 | Good general condition when butchered. Scattered, moderately extensive tuberculous lesions on autopsy. |
| 2 mg into jugular vein | 101 | Mar. 8 | Control | | | | 22 | Died of miliary tuberculosis. |
| | 102 | Apr. 15 | Control | | | | 20 | Died of miliary tuberculosis. |
| | | 1928 | | | | | | |
| | 3 | Aug. 8 | Jugular vein | 50 | 51 | 122 | 50 | Died of tuberculous pneumonia. |
| | 4 | Aug. 23 | Jugular vein | 50 | 36 | 122 | 110 | Good general condition when butchered. Lungs, thoracic nodes, and spleen studded with small tubercles. |
| | 103 | Aug. 28 | Control | | | | 32 | Died of miliary tuberculosis. |
| | 104 | Sept. 3 | Control | | | | 27 | Died of miliary tuberculosis. |
| | | 1927 | | | | | | |
| | 5 | Sept. 11 | Dewlap | 100 | 6 | 176 | 120 | Remained in fair general condition until butchered. Moderate tuberculous lesions. |
| 50 mg under skin of neck | 6 | Dec. 19 | Dewlap | 100 | 3 | 80 | 70 | Remained in fair general condition until butchered. Moderate tuberculous lesions. |
| | 105 | Dec. 9 | Control | | | | 68 | Killed *in extremis*. Massive tuberculous lesions. |
| | 106 | Dec. 23 | Control | | | | 71 | Killed *in extremis*. Massive tuberculous lesions. |

The results from the intrajugular injection of 2 mg of virulent tuberculosis culture 271 into the 2 calves that had been vaccinated by the intravenous injection of 1 gram of heat-killed tubercle bacilli are given in table 4.

TABLE 4

RESULTS FROM THE INTRAVENOUS INFECTION OF CALVES VACCINATED WITH HEAT-KILLED TUBERCLE BACILLI

| No. | Born | Intervals in days | | | Remarks |
|---|---|---|---|---|---|
| | | Birth to vaccination | Vaccination to infection | Infection to autopsy | |
| *A* | Aug. 30, 1928 | 29 | 122 | 29 | Died of miliary tuberculosis. |
| *B* | Aug. 31, 1928 | 28 | 122 | 30 | Died of miliary tuberculosis. |

NOTE.—Calves *A* and *B* were infected at the same time and in the same way as calves 3, 4, 103, and 104 (table 3). In addition to these controls, calf 94 vaccinated with 50 mg BCG intravenously and calf 96 vaccinated with 1 gram of heat-killed tubercle bacilli intravenously were protected from infection for 5 months and then slaughtered. No indications of tuberculosis were found in them on autopsy 150 days after vaccination.

The results on calves *A* and *B*, which had been vaccinated with heat-killed tubercle bacilli, support the findings of many investigators that the inoculation of dead bacilli in experimental animals yields little or no immunity to tuberculosis. Webb[78] recently reviewed this controversy and pointed out that a large majority of those who have investigated the subject are of the opinion that use of the living bacilli is necessary to develop an appreciable resistance to tuberculosis.

*Case Histories of Calves Infected Intravenously.*—Following are the case histories of the 4 calves vaccinated with BCG which were infected intravenously, as shown in table 3:

No. 1. Steer calf, ear tag 22 (see table 3) born on March 22, 1927, was vaccinated in the dewlap on March 30, 1927, with 100 mg BCG serial 27. This serial was made from a 20-day growth of the ninth transplant on potato of the culture received from Calmette on July 10, 1926. A round, hard thickening of the subcutaneous tissue of the dewlap developed, which measured 35 mm in diameter on May 23, 1927. It became impalpable a few weeks later. On August 17, 1927, 140 days after vaccination, this calf was injected in the right jugular vein with 2 mg of virulent bovine tubercle bacilli from Culture No. 271, suspended in 5 cc of physiological salt solution. The animal remained apparently normal and was in pasture with the tuberculous herd from August 17 to December 31. It was butchered on January 2, 1928, 138 days after intravenous infection.

Autopsy: The general condition was good. No macroscopic lesions of tuberculosis could be found, the lymph nodes, lungs, and other viscera being apparently normal. Stained smears from the lungs and various lymph nodes failed to show any acid-fast bacilli. Guinea pigs injected with submaxillary, atlantal, retropharyngeal, and mesenteric lymph nodes and spleen pulp failed to develop tuberculosis, while guinea pigs inoculated with a mixture of gastrohepatic, bronchial and mediastinal, and precrural and prescapular lymph nodes developed generalized tuberculosis.

Comment: The absence of macroscopic lesions but the presence in some of the apparently normal lymph nodes of virulent tubercle bacilli is in accordance with the publications of Calmette.

No. 2. Steer calf, ear tag 33 (see table 3), born on April 18, 1927, was vaccinated in the dewlap on April 22, 1927, with 100 mg BCG serial 35. This serial was made from a 23-day growth of the tenth transplant on potato of the culture received from Calmette on July 10, 1926.

A round, hard swelling developed at the point of injection in the dewlap, which was 40 mm in diameter on March 21, 1928.

On August 17, 1927, 117 days after vaccination, this calf was injected in the right jugular vein with 2 mg of virulent bovine tubercle bacilli from culture No. 271 in the same manner as calf No. 1. The animal remained apparently normal and was pastured with the tuberculous herd of calves until March 2, 1928, when it was placed in an isolated pen. At this time, two hard, round subcutaneous nodules, each about 20 mm in diameter, were observed on the side of the neck near the point of intravenous injection. On April 21, these had each increased to a diameter of about 40 mm. The anterior of these was in the center of the jugular groove at the point of intravenous injection, and the posterior nodule was 60 mm anterior and 40 mm above the upper end of the left prescapular lymph node. On April 9, 1928, the posterior nodule was removed surgically and was found to be a thin, tough-walled abscess, containing soft, buff-colored glutinous pus which, on microscopic examination, was seen to consist of amorphous cell detritus. The macroscopic appearance of the abscess was more typical of pyogenic than of tuberculous infection. However, numerous acid-fast bacilli were found in smears. Inoculation of the pus, on April 9, 1928, resulted in generalized tuberculosis in 3 guinea pigs and a rabbit.

The surgical wound in the neck of the calf healed completely by granulation, and only a slight scar was visible after 6 weeks. The anterior nodule on the neck was left undisturbed, but it did not appear to grow any after April 9.

The calf remained in good general condition and was butchered on June 21, 1928, 309 days after its intravenous infection.

Autopsy: The general condition was good. A thickening was palpable through the skin, 50 mm above the superior end of the left prescapular lymph node. On section, a dirty gray homogenous connective tissue formation, somewhat resembling lymph-node tissue, was observed involving the inner layers of the skin, the subcutaneous tissue, and the muscle. The neoplasm measured 40 x 30 x 20 mm. Portions of this lesion were injected into guinea pig No. 616, which was gassed on August 20, 1928, and was found to be negative for tuberculosis.

In the left jugular furrow, near the point of intravenous inoculation, was a round, smooth, subcutaneous abscess, 40 mm in diameter, consisting of a firm connective tissue wall, 5 mm thick. The pus content was viscid, yellowish-green, and odorless. Acid-fast bacilli were demonstrated in smears from this pus.

In the subcutaneous tissue between the left ischial tuberosity and the anus was a round, smooth, hard abscess, 50 mm in diameter, similar in structure to the abscess in the jugular furrow. Portions of this lesion were injected into guinea pig No. 619, which was gassed on July 23, 1928, and showed generalized tuberculosis.

An area of chronic bronchopneumonia, 50 mm in diameter, was present in the posterior tip of each diaphragmatic lobe of the lung. Large numbers of lung worms (*Dictyocaulus viviparus*) were seen in the adjacent bronchial tubes.

A portion of the apparently normal spleen was injected into guinea pig No. 615, which was gassed on August 20, 1928, and found to be negative for tuberculosis.

The left bronchial lymph node contained a caseous area, 5 mm in diameter. The cut surface of the right bronchial lymph node showed a red area, 6 mm in diameter. The 2 bronchial lesions and portions of the apparently normal mediastinal lymph nodes were injected into guinea pig No. 620, which was gassed on July 23, 1928, and showed generalized tuberculosis.

In the center of the mesenteric chain of lymph nodes were 2 caseous areas, about 5 mm in diameter, each surrounded by a thin connective tissue wall. Acid-fast bacilli were abundant in smears. Portions of these mesenteric lesions were injected into guinea pig No. 618, which was gassed on August 16, 1928, and showed generalized tuberculosis. Adjacent to the gastrohepatic lymph nodes was an abscess, 30 x 20 x 20 mm, containing inspissated pus. This pus was injected into guinea pig No. 617, which was gassed on August 16, 1928, and showed generalized tuberculosis.

The well-marked tuberculous lesions observed in vaccinated calf No. 2 are similar to the cases described by Tzeknovitzer[71, 72] in the Third Report of the Ukrainian Commission and indicate that some modification of Calmette's general conclusions is justified. It is noteworthy that tuberculous abscesses appeared in this calf about 7 months after intravenous infection and, on autopsy 10 months after infection, well-marked tuberculous lesions were visible in various parts of the body. The location and appearance of these, together with their high virulence for guinea pigs and a rabbit, justified the conclusion that the virulent bacilli injected intravenously on August 17, 1927, had remained alive in the tissues and after a time had resulted in the production of well-marked lesions. Those on the neck probably resulted from a few bacilli lodged in the subcutaneous and intramuscular tissue when the needle was being withdrawn from the jugular vein after the intravenous injection on August 17, 1927, but these lesions did not become palpable until March, 1928. Visible evidence was obtained that they were gradually increasing in size between March 2 and April 7. After the surgical removal of one of these, no increase in size was noted in the remaining nodule.

Calf No. 2 was associated at pasture with other tuberculous cattle from August 17, 1927, to March 2, 1928, and it is not certain that all of its tuberculous lesions were the result of intravenous infection. The caseation in the mesenteric and bronchial lymph nodes may have resulted either from tubercle bacilli ingested with the food or from the intravenous injection. The tuberculous abscesses in the ischial and gastrohepatic regions were unusual in location and appearance and are believed to have resulted from the intravenous injection of virulent bacilli.

No. 3. Steer calf, ear tag 92 (see table 3), born August 8, 1928, at Millbrae and brought to Berkeley September 20, was negative to intradermic tuberculin test given September 21, and was injected in the left jugular vein with 50 mg of BCG suspended in 2.5 cc of diluent on September 28. The animal remained apparently normal except for irregularities in temperature, which during the 2 weeks following the injection ranged from 102° to 105° F. Similar fluctuations in temperature also occurred in nonvaccinated control calves kept with this animal. The calf grew normally and on January 28, 1928, was subjected to infection by injection in the left jugular of 2 mg of virulent bovine tubercle bacilli from culture 271. The animal remained normal in general appearance until February 19, after which time it developed a chronic cough, showed some anorexia and marked hyperthermia. It gradually became weaker and died on March 19, 1929.

Autopsy: The carcass was greatly emaciated. Muscular tissues shrunken. Intermuscular tissue was edematous. The lungs were studded with gray-white nodules, varying from 3 to 12 mm in size. The lungs felt like a bag full of peas. In many places the diaphragmatic lobes were so thickly studded that the nodules were confluent, and when small chunks of these lobes were dropped into water, they immediately sank. The tips of the apical and cardiac lobes were not so thickly studded, there being spaces of normal tissue visible. Some of the larger nodules had caseous centers. The pulmonary lymph nodes were enlarged about five times and showed necrotic foci involving most of their tissue. There was considerable hyperplasia of the costal pleura. Other than this, the tissues were apparently normal, except for post-mortem changes. Acid-fast bacilli were abundant in the thoracic lesions, but none could be found in the apparently normal abdominal organs.

The chief difference between the lesions in No. 3 and in other animals of this group which had died previously was as follows:

The nonvaccinated controls and the 2 calves treated with heat-killed bacilli died of acute miliary tuberculosis, particularly of the lungs, but also involving organs in the abdominal cavity. Calf No. 3 appeared to have died of tuberculous pneumonia, the lesions being confined to the thorax. The tubercles, while very numerous in the lungs, were not as numerous as in the nonvaccinated calves. However, in calf No. 3 the tubercles were 3 to 12 mm in size, while in the control calves and in calves *A* and *B* they were all miliary in type, 2 to 3 mm.

No. 4. Steer calf, ear tag 94 (see table 3), born August 23, 1928, at Millbrae and brought to Berkeley September 20, was negative to intradermic tuberculin test given September 21 and was injected in the left jugular vein with 50 mg of BCG suspended in 2.5 cc of diluent on September 28. The animal remained apparently normal except for slight irregularities in temperature. On January 28, 1929, this calf received intravenously 2 mg of virulent tubercle bacilli from culture 271 in the same manner as calf No. 1.

During the 3rd and 4th weeks following, the animal had a persistent cough, rapid respiration, and occasionally slight discharge of blood from the nostrils. Its temperature showed relatively slight variations and it remained otherwise in apparently normal condition until slaughtered on May 18, 1929.

Autopsy: The carcass was well nourished. The lungs were sprinkled with yellow nodules 2 to 4 mm in diameter. The thoracic lymph nodes were enlarged three to five times and thickly studded with hard necrotic nodules. The spleen was studded throughout with white nodules 1 to 2 mm in diameter. The average distance between the nodules was about 30 mm. Acid-fast bacilli were demonstrated in the lesions indicated above.

*Case Histories of Calves Infected Subcutaneously.*—Following are the case histories of the 4 calves which were infected subcutaneously as shown in table 3:

No. 5. Steer calf, ear tag 69 (see table 3), born on September 11, 1927, was vaccinated in the dewlap on September 17, 1927, with 100 mg BCG, serial No. 62. It was reared in an environment believed to be free from bovine tubercle bacilli

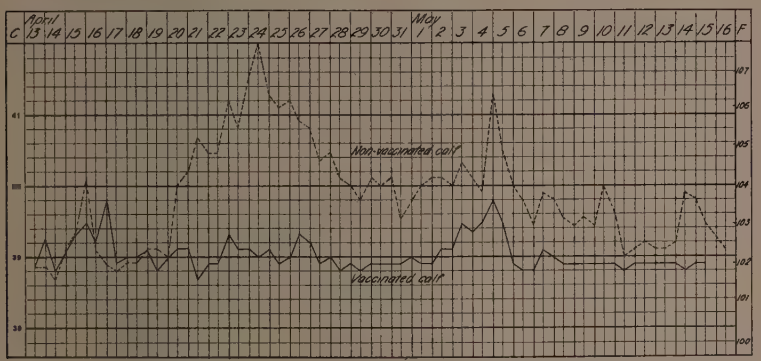


Fig. 1. Temperatures of calves Nos. 5 (vaccinated) and 105 (control), injected subcutaneously with 50 mg of virulent tubercle bacilli, March 12, 1928. The dotted line indicates the temperature of the nonvaccinated control.

until March 12, 1928. One hundred and seventy-seven days after vaccination, the animal was injected under the skin of the neck on the left side with 50 mg of virulent bovine tubercle bacilli from culture No. 271. (See page 322 for description of this culture.)

The animal remained apparently normal (fig. 1) except for the swelling at the point of injection which gradually developed and which on May 6, 1928, measured 90 x 50 x 35 mm. After this no increase in size was apparent (see fig. 4, p. 386). The calf was killed on July 10, 1928, 120 days after infection, in order to compare the autopsy findings with control animal 105.

Autopsy: The general condition was good. At the point of inoculation of the virulent bacilli was a nodule 70 x 35 x 20 mm, occupying subcutaneous tissue only and containing creamy tenacious pus. Adjacent and located 10 cm apart between the superficial neck muscles, were two abscesses well encapsulated, 20 x 10 mm, containing greenish-yellow granular pus. Nearby was an abscess similar in character measuring 10 x 10 mm.

A 120-mm linear abscess within the neck muscle tissue extended from the point of injection to another subcutaneous nodule, which was irregular in shape, and about 50 x 50 mm. The wall of this linear abscess was 30 mm thick.

The left prescapular lymph node was slightly enlarged and studded throughout with caseated (mostly soft) areas, varying in diameter from 3 to 10 mm. In the left bronchial lymph node was a necrotic point 0.5 mm in size. In the anterior mediastinal lymph node were two 2-mm nodules. A microscopic examination showed numerous acid-fast organisms in the abscesses in the neck. An occasional acid-fast organism was seen in smears from the left prescapular lymph node. Guinea pigs were injected as follows:

| Source of material | Guinea pig No. | Date killed 1928 | Results |
|---|---|---|---|
| Head and mesenteric nodes | 667 | Sept. 8 | Negative |
| Lung and thoracic nodes | 668 | Aug. 13 | Moderate tuberculosis |
| Muscle at point of injection | 669 | Aug. 13 | Generalized tuberculosis |

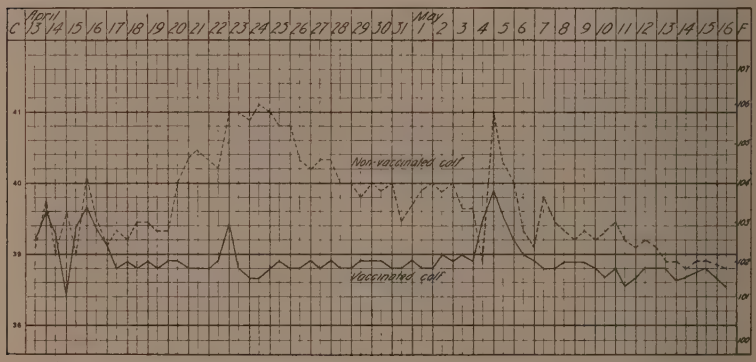


**Fig. 2.** Temperatures of calves Nos. 6 (vaccinated) and 106 (control), injected subcutaneously with 50 mg virulent tubercle bacilli, March 12, 1928. The dotted line indicates the temperature of the nonvaccinated control.

No. 6. Steer calf, ear tag 75 (see table 3), born on December 19, 1927, was vaccinated in the dewlap on December 22, 1927, with 100 mg BCG. It was reared in an environment believed to be free from virulent tubercle bacilli until March 12, 1928, 81 days after vaccination, when it was injected subcutaneously in the left side of the neck with 50 mg of virulent tubercle bacilli from culture No. 271. The animal remained apparently normal except for a subcutaneous swelling at the point of injection, which measured 90 x 50 x 35 mm on May 6, 1928. (See fig. 5.) The temperature remained fairly normal (fig. 2). Slaughtered on May 21, 1928, 70 days after infection, for the purpose of comparing with steers 105 and 106.

Autopsy: The general condition was good. In the subcutaneous tissue of the neck, at the point where virulent tubercle bacilli had been injected, was a flat, thin-walled abscess, 100 x 50 x 35 mm, containing soft, creamy pus. Smears showed an occasional acid-fast bacillus. The adjacent prescapular lymph node was slightly enlarged (size 100 x 35 x 35 mm) and its cut surface was studded with calcareo-caseous nodules, 1 to 5 mm in diameter. Several nodules each about 2 mm in diameter were found in the parenchyma of the spleen, and guinea pig No. 607 was

inoculated with some of these nodules. It was gassed on July 12, 1928, and found to have generalized tuberculosis.

The lymph nodes, other than the left prescapular, were apparently normal throughout the viscera and body. Bronchial and mediastinal lymph nodes were injected into guinea pig No. 606. It was gassed on July 12, 1928, and showed generalized tuberculosis. Portions of the apparently normal mesenteric lymph nodes were injected into guinea pig No. 604, and the lung tissue into No. 605. They were gassed on July 17, 1928, and both found free from tuberculosis.

No. 105. Steer calf, ear tag 191 (see table 3), born on December 9, 1927, was reared on an environment believed to have been free from virulent bovine tubercle bacilli. It was negative to an intradermic test with 0.2 cc B. A. I. tuberculin on March 1, 1928. It was used as a nonvaccinated control for steers 5 and 6, by the subcutaneous injection, on March 12, 1928, of 50 mg of virulent bovine tubercle bacilli from culture No. 271. The animal developed a swelling at the point of injection, which rapidly increased in size until May 6, 1928, at which time it measured 160 x 120 x 70 mm, and the adjacent prescapular lymph node was 230 x 130 x 120 mm (see fig. 6). After this date these swelling increased slowly. On April 20, the animal's temperature rose to 104° F and fluctuated between 104° and 106° F for a week, after which it gradually returned to normal by May 6 (fig. 1).

The animal was depressed in appetite and activity during the period of high temperature. About May 1, the abscess in the side of the neck ruptured through the skin and continued to discharge until date of slaughter. The animal appeared to be in such an advanced stage of tuberculosis that it was decided to slaughter it on May 19, 1928, 68 days after infection.

Autopsy: The general condition was fair, except for the massive lesions on the left side of the neck. The abscess at the point of injection consisted of a mass of caseocalcareous tuberculous tissue, 200 x 190 x 80 mm, in the center of which was a spot of soft, putrid, caseous liquefaction, 50 x 40 x 40 mm. An edematous mass of loose connective tissue surrounded the left prescapular lymph node. Including the node, it measured 230 x 130 x 120 mm. The lymph node itself was enlarged and changed into a tuberculous mass, 190 x 110 x 110 mm, in the center of which was a spot of soft, caseous liquefaction, 40 mm in diameter.

The lungs, liver, and spleen were studded throughout with grey nodules, ranging from 2 to 10 mm in diameter. The distance between the tubercles in the lung ranged from 5 to 20 mm. Eighty-one such nodules were observed on the diaphragmatic surface of the liver, which illustrates about the density of those throughout the liver and spleen.

The thoracic, axillary, pectoral, and left radiohumeral lymph nodes were enlarged several times and their cut surfaces were thickly studded with caseocalcareous nodules. Each of the cervical and gastrohepatic lymph nodes contained several caseous nodules, and a caseous nodule was found in one of the lymph nodes of the mesenteric chain.

A number of pendulous nodules, ranging from 5 to 10 mm in diameter, were observed on the omentum and serous surfaces of the mesentery and intestines. Some of these nodules had caseous centers. Acid-fast bacilli were demonstrated in smears from various lesions, including the lungs, kidney, and the one mesenteric node mentioned.

No. 106 Steer calf, ear tag 193 (see table 3), born on December 23, 1927, was reared in an environment believed to have been free from virulent bovine tubercle

bacilli. It was negative to an intradermic test with 0.2 cc B. A. I. tuberculin on March 1, 1928. It was used as a nonvaccinated control for steers 5 and 6 on March 12, 1928, by the subcutaneous injection of 50 mg of virulent bovine tubercle bacilli from culture No. 271.

The animal developed a swelling at the point of injection in the left side of the neck, which rapidly increased in size until May 6, 1928, at which time it measured 180 x 120 x 80 mm, and the adjacent prescapular lymph node was 200 x 100 x 100 mm (see fig. 7). After that date, these swellings increased slowly. On April 20 the animal's temperature rose to 104.0° F and fluctuated between 104.0° and 107.6° F for 10 days, after which it gradually returned to normal, which it reached by May 6 (fig. 2). It was depressed in appetite and activity during the period of high temperature. The animal developed such an advanced stage of tuberculosis that it was killed on May 22, 1928.

Autopsy: The general condition was fair. In the subcutaneous tissue of the neck, at the point where virulent tubercle bacilli had been injected, was a solid caseocalcareous mass, 185 x 150 x 90 mm. The adjacent prescapular lymph node apparently measured 210 x 110 x 100 mm, but when cut across, it was noted to be 100 x 70 x 70 mm, and the balance of the swelling was edematous connective tissue. The cut surface of the lymph node was caseocalcareous and firm.

The liver, lungs, and spleen were studded throughout with white and pinkish-white nodules, ranging from 1 to 5 mm in diameter. Two hundred and fifty such nodules were visible on the diaphragmatic surface of the liver. Acid-fast bacilli were abundant in the neck lesion and liver lesions. Five pendulous nodules, each 5 mm in diameter, were observed on the surface of the right costal pleura.

The omentum and mesentery were studded with pendulous pinkish-white nodules, about 5 mm in diameter. It was estimated that there was one for each square decimeter of surface. The bronchial, mediastinal, and gastrohepatic groups of lymph nodes were enlarged five to ten times and their cut surfaces were thickly studded with caseous nodules, 1 to 3 mm in diameter. The following groups of lymph nodes were slightly enlarged and each node contained one to seven caseous nodules, from 1 to 3 mm in diameter: the submaxillary, atlantal, retropharyngeal, left axillary, left scapulohumeral, and both precrurals. Smears from the retropharyngeal lymph nodes showed acid-fast bacilli. About one-third of the nodes of the mesenteric chain showed on their cut surfaces 2 to 5 caseous nodules ranging from 1 to 5 mm in diameter.

Some of the spleen nodules were injected into guinea pig No. 608, which was gassed on July 12, 1928 and showed generalized tuberculosis.

In the 4 calves (Nos. 5, 6, 105 and 106) that were infected subcutaneously it was noted that the most rapid development of the local lesions occurred during the first 3 weeks following the subcutaneous infection and that during this time hyperthermia and anorexia occurred in the controls. The controls showed a distinctly greater susceptibility as evidenced by the temperatures shown in figures 1 and 2, the general symptoms of the animals, and particularly the tuberculous lesions found on autopsy. It is believed that the 2 controls would have died of tuberculosis in a few days if they had not been slaughtered for humane reasons. On the other hand, the lesions

in the 2 vaccinated calves were so limited in extent that it appears probable that they would have lived indefinitely.

*Discussion of the Results of Intravenous and Subcutaneous Infection as Shown in Tables 3 and 4.*—The relatively high virulence of test culture No. 271 for cattle was demonstrated by the death from miliary tuberculosis of the 4 nonvaccinated control calves and the 2 calves treated with killed tubercle bacilli in 20 to 32 days, after intravenous infection with a 2-mg dose. The appearance of the tissues of these calves were all about the same. The lungs of each were filled with grey nodules 1 to 4 mm in size and there was a sprinkling of similar nodules throughout the liver and kidneys. Acid-fast bacilli were demonstrated in smears and in sections and the macroscopic and microscopic findings were typical of acute miliary tuberculosis. There was no distinguishable difference between the lesions of the nonvaccinated controls and those which had received the heat-killed tubercle bacilli.

On account of need for brevity a detailed description and history of the control calves infected intravenously has been omitted from this paper.

The fact that the 2 calves (Nos. 1 and 2) which had been vaccinated in the dewlap lived on for several months after intravenous infection without showing any outward evidence of tuberculosis, while the 2 controls (Nos. 101 and 102) died of miliary tuberculosis in 20 and 22 days respectively is in accordance with the results of experiments by Calmette and Guérin.[20] Also, the fact that calves (Nos. 3 and 4) that had been vaccinated intravenously with BCG both showed clinical symptoms within a month after infection supports the claims of Calmette that subcutaneous vaccination with BCG is more effective than intravenous vaccination in creating resistance to tuberculosis.

The lesions found at autopsies of Nos. 3 and 4 indicated that these calves were more resistant than the controls, and the fact that they lived longer than the controls would also show that some resistance had been produced by the BCG vaccine.

The injurious effect of the virulent bacilli on the controls Nos. 105 and 106 contrasted with the distinctly less marked pathologic effect on 2 vaccinated calves Nos. 5 and 6, indicates that the BCG had conferred resistance to subcutaneous infection. This experiment acquires added significance when compared with the results obtained in the calves infected in other ways, as described later in this paper.

Cobbett[26] summarized the data of the British Royal Commission and the German Gesundheitsamt and called attention to the marked

differences in the power to resist tuberculous infection which occur in individual calves, showing that such differences play a more important part when virulent bacilli in doses of moderate size (10 mg) are injected subcutaneously than when larger doses (50 mg) are given in the same manner. According to Cobbett the workers in the German Gesundheitsamt noted variations in susceptibility to tuberculosis among calves, and they recommend that when but a single calf is used in typing a strain and unexpected results are obtained, the experiment should be repeated on another calf.

## THE EFFECTS OF FEEDING TUBERCULOUS TISSUES AND MILK TO VACCINATED AND CONTROL CALVES

After weighing carefully the arguments for and against natural and artificial infection in an effort to determine the protective efficiency of the BCG culture, it was decided that much more can be learned from artificial feeding infection with tuberculous tissues than by the somewhat haphazard exposure to infected cattle or environs, and since the vaccination trials in the Williamson herd involved natural infection, efforts at Berkeley and Davis were concentrated on artificial infection with tuberculous material.

Fifty vaccinated calves and an equal number of unvaccinated controls have been subjected to infection by feeding tuberculous material. The tissues were ground in a meat-grinding machine, thoroughly mixed with milk, and except that fed to animals in table 6, was strained through several layers of cheese cloth. Each dose was fed separately to each calf in the manner described in the footnotes to the tables. Animals listed in table 6 were given the ground, unstrained tuberculous tissue in milk.

The tissues were obtained from cattle condemned at an abattoir or from guinea pigs and rabbits inoculated with bovine tuberculous materials. In addition, 4 vaccinated calves and 4 controls were fed on milk from the tuberculous udders of cows No. 600 or No. 2171 (see figs. 9 and 10).

The sources of the tuberculous tissue, the respective amounts fed each of the 100 calves, and the results of the microscopic and biologic control tests on each feeding are given in table 5.

Before giving the first infecting feeding, the vaccinated calves and the nonvaccinated controls, which had been reared together at the dairy in Berkeley in an environment protected as much as possible from virulent tubercle bacilli, were transferred to an isolated barn

TABLE 5

SOURCES AND AMOUNTS OF TUBERCULOSIS TISSUES AND BACILLI USED TO INFECT CALVES BY FEEDING

| Label numbers of calves fed | Date collected | Description of material | Date fed | Dose fed each calf | | Guinea-pig control | |
|---|---|---|---|---|---|---|---|
| | | | | Grams of tissue* | Estimated acid-fast bacilli† | Mg tissue injected‡ | Result |
| | 1926 | | 1926 | | | | |
| | Sept. 1 | Portions of tuberculous lymph nodes, lungs, and livers of 17 cattle, condemned at an abattoir | Sept. 2 | 80 | § | § | + |
| 7–10, and 107–110 | Sept. 2 | Tissues from a guinea pig injected with bovine lesions | Sept. 6 | 6 | § | § | + |
| | Sept. 26 | Tissues from guinea pigs injected with bovine lesions | Sept. 27 | 4 | § | § | + |
| | Oct. 23 | Active tubercles from the pleura, spleen, and omentum of a cow | Oct. 25 | 37 | § | 500.0 | + |
| 7–10, 107–110, *C*, *D*, *E*, and *F* | Nov. 26 | Caseous pus from tuberculous cattle tissues condemned at an abattoir | Nov. 27 | 80 | § | 250.0 | + |
| | | | Nov. 29 | 80 | § | 25.0 | + |
| | 1927 | | 1927 | | | | |
| | Aug. 12 | Tuberculous tissue from the lungs, costal pleura and lymph nodes of 2 aged cows and one 7-month calf | Aug. 13 | 10 | 500,000 | 50.0 | + |
| | | | Aug. 15 | 10 | 500,000 | 50.0 | + |
| | Aug. 16 | Tuberculous tissue from the lungs, costal pleura, and lymph nodes of an aged cow | Aug. 17 | 2 | 690,000 | ..... | ..... |
| | | | Aug. 19 | 2 | 690,000 | 0.5 | + |
| 11–44 | | | Aug. 22 | 2 | 690,000 | 1.25 | + |
| 111–144 | | | Aug. 23 | 2 | 690,000 | 1.0 | + |
| | Aug. 26 | Tuberculous tissue from 2 rabbits and 2 guinea pigs | Aug. 27 | 2 | 1,320,000 | 1.0 | + |
| | Sept. 1 | Tissues from lungs and lymph nodes of a range steer | Sept. 2 | 2 | 850,000 | 1.0 | + |
| | Sept. 7 | Lung of calf No. 101, which died of miliary tuberculosis (see table 3) | Sept. 8 | 0.5 | 1,890,000 | 0.5 | + |
| | | | Sept. 9 | 0.5 | 1,890,000 | 0.5 | + |
| | Sept. 10 | Lymph node tissue, calf No. 101 | Sept. 11 | 2 | 960,000 | 2.0 | + |
| | | | Sept. 12 | 2 | 960,000 | 2.0 | + |
| 30–44 and 130–144 | Sept. 13 | Lung of calf No. 102, died of miliary tuberculosis, (see table 3) | Sept. 14 | 5 | 1,950,000 | 5.0 | + |
| | | | Sept. 16 | 5 | 1,950,000 | 5.0 | + |
| | Sept. 13 | Lung and lymph nodes, calf No. 102 | Sept. 18 | 0.3 | 2,000,000 | 0.3 | + |
| | | | Sept. 19 | 0.3 | 2,000,000 | 0.3 | + |

TABLE 5—(*Continued*)

| Label numbers of calves fed | Date collected | Description of material | Date fed | Dose fed each calf: Grams of tissue* | Dose fed each calf: Estimated acid-fast bacilli† | Guinea-pig control: Mg tissue injected‡ | Guinea-pig control: Result |
|---|---|---|---|---|---|---|---|
| | Sept. 23 | Tuberculous viscera from guinea pigs that had been inoculated with the feeding, August 13, this table | Sept. 24 | 1.0 | 2,040,000 | 1.0 | + |
| | | | Sept. 28 | 0.5 | 1,020,000 | 0.5 | + |
| | | | Sept. 29 | 0.5 | 5,850,000 | 0.2 | + |
| | | | Sept. 30 | 0.5 | 5,850,000 | 1.0 | + |
| 30–44 and 130–144 (*Cont.*) | Sept. 28 | Caseous material from tuberculous lesions in the lungs and lymph nodes of an aged cow | Oct. 1 | 0.5 | 5,850,000 | 1.0 | + |
| | | | Oct. 2 | 0.5 | 5,850,000 | 0.1 | + |
| | | | Oct. 3 | 0.5 | 5,850,000 | 0.2 | + |
| | | | Oct. 4 | 0.5 | 5,850,000 | 0.1 | + |
| | | | Oct. 5 | 1.11 | 12,980,000 | 5.5 | + |
| | | | Oct. 6 | 1.11 | 12,980,000 | 5.5 | + |
| | | | Oct. 7 | 1.11 | 12,980,000 | 0.5 | + |
| | | | Oct. 8 | 1.11 | 12,980,000 | 0.005 | + |
| | Oct. 10 | Tuberculous viscera of 14 guinea pigs inoculated from feedings, Sept. 2–8, this table | Oct. 11 | 1.11 | 12,980,000 | 0.1 | + |
| | | | Oct. 12 | 1.11 | 12,980,000 | 2.0 | + |
| | Oct. 13 | Tuberculous viscera of 8 guinea pigs inoculated with bovine lesions | Oct. 14 | 1.8 | 45,000,000 | 1.8 | + |
| | | | Oct. 15 | 1.8 | 45,000,000 | 0.9 | + |
| | Oct. 15 | Tuberculous viscera of 16 guinea pigs inoculated with feedings, Sept. 9–14, this table | Oct. 16 | 4.8 | 67,500,000 | 0 24 | + |
| 40–44 and 140–144 | | | Oct. 17 | 4.8 | 67,500,000 | 0 24 | + |
| | Oct. 18 | Tuberculous viscera of 21 guinea pigs inoculated with feedings, Sept. 14–19, this table | | | | 0 000034 | – |
| | | | Oct. 19 | 6.8 | 73,100,000 | 0 0034 | + |
| | Oct. 19 | Viscera of 3 guinea pigs and 1 rabbit which had died of tuberculosis after injection with bovine lesions | Oct. 20 | 11.0 | 3,500,000,000 | 0.000005 | + |
| | **1928** | | **1928** | | | | |
| 49–54 and 149–154 | July 6 | Caseous and cellular tissue from tuberculous lesions in the lungs and thoracic lymph nodes of 2 cows condemned at an abattoir | July 7 | 42.0 | 210,000,000 | 0 00001 | + |
| 55–59 and 155–159 | July 6 | Caseous and cellular tissue from tuberculous lesions in the lungs and thoracic lymph nodes of 2 cows condemned at an abattoir | July 7 | 14.0 | 70,000,000 | 1.0 | + |
| | | | July 9 | 14.0 | 70,000,000 | 1.0 | + |
| | | | July 11 | 14.0 | 70,000,000 | 0 00001 | + |

TABLE 5—(*Concluded*)

| Label numbers of calves fed | Date collected | Description of material | Date fed | Dose fed each calf | | Guinea-pig control | |
|---|---|---|---|---|---|---|---|
| | | | | Grams of tissue* | Estimated acid-fast bacilli† | Mg tissue injected‡ | Result |
| 60 and 160 | Aug. 8 | Caseous and cellular material from tuberculous lesions in the costal pleura or in the lungs and lymph nodes of a steer, a cow, and 2 calves condemned at an abattoir | Aug. 10 | 60 | 1,572,000,000 | 0.00006 | + |
| | | | Aug. 12 | 60 | 1,572,000,000 | 0.3 | + |
| | Aug. 13 | Tuberculous lesions from 11 guinea pigs inoculated with bovine lesions | Aug. 14 | 30 | 107,000,000 | 0.00001 | + |

* This represents the weight of tuberculous tissue after grinding but before straining through several thicknesses of cheese cloth. The actual amount of original tissue in each dose is less than one-third of this weight.

† Made by suspending 10 or 20 grams of thoroughly ground and mixed tuberculous tissue in a measured quantity of physiological sodium-chloride solution; then estimating according to the technique for the direct microscopic counting of bacteria in milk.[4] The estimate was only comparative. The number of organisms consumed was far greater than indicated in this column, since the character of the tissue and the method of estimating did not lend themselves to more definite determination of number of organisms.

‡ The inoculum for the guinea pig was a portion of the ground and strained tuberculous materials suspended in milk as fed to the calves and the weight is given in terms of ground but unstrained material.

§ In the feedings indicated by this mark (§), acid-fast bacilli were abundant, but no attempt was made to estimate the number of bacilli or the milligrams of tissue injected into control guinea pigs.

and corrals at Berkeley or to a farm at Davis where no other cattle had been kept for several years.

Calmette[12] has stated that large, frequently-repeated doses of tubercle bacilli given by the mouth will produce pronounced lesions in calves, but, if only one dose is given and the animal is then protected from infection for a time, only slight lesions are produced which render the animal resistant to further infection.

In order to avoid as far as possible this supposed immunizing effect induced by feeding small doses of tubercle bacilli at long intervals, the feeding experiments at the California station have been with large doses at frequent intervals.

An attempt was also made to determine if the prolonged feeding of large doses would produce more extensive lesions than a few feedings. The significance of this attempt will be evident by comparing the graphic symbols for tuberculous lesions in the nonvaccinated control animals listed in table 6. Controls 107–110 received 7 infecting feedings, the dates being September 2, 6, 27, and 29, October 25, and November 27 and 29, while controls *C* and *D* received only the feedings on the last 2 dates. The results in these 2 calves alone are

TABLE 6

RESULTS FROM FEEDING* TUBERCULOUS TISSUE TO CALVES VACCINATED IN THE DEWLAP WITH 50 MG BCG AND TO CONTROL CALVES

| No. | Born | Intervals in days | | | Number of infecting feedings‡ | General condition on slaughter | Tuberculous lesions† | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to vaccination | Vaccination to first infecting feeding | First infecting feeding to autopsy | | | Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | Other lesions | |
| 7 | Apr. 23, 1926 | 3 | 129 | 123 | 7 | Good | | | | | / | | | No caseation. One mesenteric lymph node hemorrhagic and guinea pigs inoculated from it became tuberculous. No dewlap lesion. |
| 8 | Apr. 26, 1926 | 4 | 108 | 123 | 7 | Good | | | | | | | | No lesions except abscess in dewlap, 70 x 30 mm. |
| 9 | May 7, 1926 | 1 | 127 | 154 | 7 | Good | | | | | / | | | Caseous nodule, 4 x 4 mm in a mesenteric lymph node. Guinea pigs inoculated developed tuberculosis. Connective tissue thickening in dewlap, 20 x 5 mm. |
| 10 | May 18, 1926 | 3 | 115 | 154 | 7 | Good | / | | | | × | | | Four 2-mm nodules in retropharyngeal. Many caseocalcareous mesenteric lymph nodes. Guinea pigs became tuberculous. No dewlap lesion. |
| 107 | Feb. 24, 1926 | Control | | 123 | 7 | Good | × | | | | | | | Caseocalcareous retropharyngeal 110 mm. Submaxillary 40 mm. Acid-fast bacilli numerous. |
| 108 | Mar. 8, 1926 | Control | | 123 | 7 | Poor | | | | | × | × | | Many caseocalcareous lesions in mesenteric and ileocecal nodes. Acid-fast bacilli numerous. |
| 109 | Mar. 13, 1926 | Control | | 154 | 7 | Good | × | | | | / | × | | Retropharyngeals enlarged 3 x and caseocalcareous. Ileocecal and colic nodes all caseocalcareous. Slight mesenteric lesions. |
| 110 | Apr. 19, 1926 | Control | | 154 | 7 | Good | | | | | / | | × | Gastric node 50 mm and 2 mesenterics caseocalcareous. 2 mesenterics hemorrhagic. Guinea pigs became tuberculous. |
| *C* | Sept. 30, 1926 | Control | | 36 | 2 | Good | \ | | | | | | | Postpharyngeal had a 35-mm caseous mass. Acid-fast bacilli present |
| *D* | Aug. 22, 1926 | Control | | 47 | 2 | Fair | × | | × | / | | | × | Cervical, mediastinal, and mesenteric caseocalcareous. One 4-mm nodule in lung. |
| *E* | July 31, 1926 | § | 28 | 36 | 2 | Poor | | × | × | × | | | | Tuberculous pneumonia evidently from inhalation of infecting feed. |
| *F* | Apr. 22, 1926 | § | 28 | 47 | 2 | Poor | × | / | | / | × | × | | Entire mesenteric chain caseocalcareous. Cervical and cecal caseocalcareous. |

* The animals were given this dose with the aid of a long-necked bottle.

† The symbols for lesions are adopted from charts used by federal meat inspectors and the names of the various groups and individual lymph nodes or lymph glands correspond in the main to those given by Buckley and Castor.[6] The adjectives bronchial, mesenteric, etc., referring to lymph nodes are, for brevity, used without the words "lymph nodes". Definitions for *slight*, *well marked*, and *extensive* have been formulated by the writers as a general guide as follows:

/ *Slight*—Caseous or caseocalcareous isolated areas less than 15 mm. in diameter.

\ *Well marked*.—A single caseous or caseocalcareous area, 15 to 50 mm in diameter. Cases having more than 1 lesion over 25 mm are recorded as extensive.

inconclusive but are significant when added to the data from the 34 control calves in tables 7*B*, 8*B*, and 9*B*.

*Discussion of the Results of Preliminary Infecting Feedings, as Given in Table 6.*—The data in this table may be summarized by stating that 7 feedings produced lesions as follows:

1. In 4 calves vaccinated with BCG, 1 extensive, 2 slight, and 1 no-lesion case.

2. In 4 nonvaccinated calves, 4 extensive-lesion cases.

In addition, 3 of the 4 control calves (*C*, *D*, *E*, and *F*) that received only the 2 last feedings on November 27 and 29 showed very extensive lesions. The fourth showed well-marked lesions in the cervicals.

The difference between the lesions in the vaccinated and the control calves listed in table 6 was not distinct enough in the writers' opinion to justify a definite conclusion regarding the existence of a protective effect from the BCG vaccination. If present, it evidently was not sufficient to prevent the penetration of virulent bacilli into the cervical or mesenteric lymph nodes and the production of caseous lesions in 2 out of 4 of the vaccinated calves. However, it was decided that further trials by this method of feeding infection might yield more definite information regarding any resistance to tuberculosis which might result from the use of BCG given subcutaneously. Accordingly feeding infection trials were carried out as indicated in tables 7–9.

*Discussion of the Results of Ten Infecting Feedings, as Given in Tables 7A and 7B.*—From an inspection of table 7*A*, it will be seen that all the calves which were infected within 25 days after the first vaccination showed well-marked or widely distributed lesions, or both, while in all the other vaccinated calves the lesions were either absent or limited to the cervical and mesenteric or colic regions. Therefore, for purposes of brevity in discussion, the calves in table 7*A* will be grouped as follows:

1. Calves 11–24 were aged 41 to 407 days at time of first feeding infection, which was given 35 to 320 days after the first vaccination. Of the 14 animals in this group, 5 were found to be apparently normal throughout, 6 showed slight, and 3 showed well-marked caseation or caseocalcification of certain lymph nodes draining the intestinal or pharyngeal regions. All of the carcasses would have been considered as suitable for food under federal meat-inspection regulation. Calf No. 25 died of intercurrent disease too soon after infection to justify consideration.

2. Calves 26–29 were aged 4 to 33 days at time of first feeding infection, which was given from 2 days before to 24 days after vaccination. Three of these had well-marked and widely distributed

## TABLE 7A

RESULTS OF TEN FEEDINGS* OF TUBERCULOUS TISSUE TO CALVES VACCINATED IN THE DEWLAP WITH 100 MG BCG

(For controls, see table 7*B*)

Feeding dates: August 13, 15, 17, 19, 22, 23, 27, September 2, 8, and 9 (For feeding details see table 5)

| No. | Born | Intervals in days | | | General condition on slaughter | Tuberculous lesions† | | | | | | | Acid-fast bacilli found in smears § | Guinea-pig tests | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to vaccination‡ | Vaccination to first infecting feeding | First infecting feeding to autopsy | | Cervical | Bronchial | Mediastinal | Mesenteric | Cecal and colic | Gastro-hepatic | Other lesions | | | |
| 11 | July 2, 1926 | 87 (225)‡ | 320 (182)‡ | 137 | Good | | | | | | | | None | − | No lesions of tuberculosis except 3 abscesses in dewlap, 40 to 100 mm. |
| 12 | Mar. 16, 1927 | 4 | 148 | 338 | Good | | | | | | | | None | − | No lesions of tuberculosis except connective tissue and caseation in dewlap, 40 x 7 mm. Chronic gastrohepatitis from wire. |
| 13 | Mar. 18, 1927 | 2 | 148 | 338 | Good | | | | | | | | None | See remarks | No lesions of tuberculosis found. Guinea pigs died of intercurrent disease. |
| 14 | Apr. 12, 1927 | 8 | 129 | 332 | Fair | | | | / | / | | | None | + | 6 caseous nodules, 2 to 5 mm in mesenteric, and 3 in cecal nodes. No dewlap lesion. |
| 15 | Apr. 2, 1927 | 5 (136) | 129 (−2) | 186 | Good | | | | / | | | | None | + | 9 caseous nodules, 1 to 2 mm, scattered through the mesenteric chain. Guinea pig positive from apparently normal retropharyngeal. Dewlap induration, 60 x 40 x 95 mm. |
| 16 | Apr. 12, 1927 | 5 (101) | 142 (46) | 323 | Good | | | | / | | | | Few | + | 2 nodules, each 2 mm, in mesenteric nodes. Connective tissue mass in dewlap, 60 x 50 x 5 mm. |
| 17 | Apr. 24, 1927 | 6 | 105 | 339 | Good | \ | | | / | | | | Few | + | 23 caseous nodules, 2 to 8 mm, scattered in mesenteric chain and 1 caseous lesion in retropharyngeal, 5 x 5 x 40 mm. |
| 18 | May 18, 1927 | 8 (41) | 79 (46) | 360 | Good | / | | | / | | | | Few | + | Retropharyngeal, 3-mm caseous nodule. 10 fresh caseous nodules, 1 to 6 mm, scattered in mesenterics. No dewlap lesion. |
| 19 | May 26, 1927 | 9 | 70 | 186 | Good | | | | \ | | | | Few | + | 40 caseous or caseocalcareous nodules, 1 to 10 mm, scattered through the mesenteric chain, and also 1 mass of caseocalcareous nodules, 40 x 20 x 20 mm. Dewlap abscess, 110 mm. |
| 20 | June 1, 1927 | 4 (76) | 70 (−2) | 351 | Good | / | | | \ | \ | | | Few | + | 5 postpharyngeal, caseous nodules, 4 to 10 mm. 11 caseous or caseocalcareous nodules, 5 to 20 mm in mesenteric and cecal nodes. No dewlap lesion. |
| 21 | June 11, 1927 | 9 | 54 | 396 | Fair | | | | | | | | None | + | No lesions of tuberculosis except connective tissue thickening in dewlap, 40 x 40 x 20 mm. Tuberculosis in guinea pigs from apparently normal mesenteric lymph nodes. |

TABLE 7B

RESULTS OF TEN FEEDINGS* OF TUBERCULOUS TISSUE IN CONTROL CALVES NOT VACCINATED

Feeding dates the same as in table 7A

| No. | Born | Intervals in days | | Tuberculous lesions† | | | | | | | | Acid-fast bacilli found in smears | Guinea-pig tests | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to first infecting feeding | First infecting feeding to autopsy | Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | Gastro-hepatic | Other lesions | | | |
| 111 | Dec. 29, 1926 | 227 | 137 | .. | .. | . | .. | .. | \ | ... | .. | ‡ | + | 2 ileocecal nodes enlarged 2 x and caseocalcareous. |
| 112 | Jan. 26, 1927 | 199 | 338 | . | .. | \ | × | . | . | . | \ | None | See remarks | Extensive tuberculosis of right lung and well-marked lesions in the left lung, caseous foci in mediastinal. Guinea pigs died of intercurrent causes. |
| 113 | Feb. 5, 1927 | 190 | 338 | . | . | . | .. | ..... | ... | ..... | . . | None | + | No visible lesions of tuberculosis. The tissue from an apparently normal mesenteric lymph node produced tuberculosis in a guinea pig. |
| 114 | Mar. 16, 1927 | 152 | 332 | / | . | | ... | \ | . | .. | ... | Many | + | 2 caseous points in a retropharyngeal, 2 and 8 mm, respectively. 5 caseous nodules in mesenteric chain, 3 to 20 mm in size. |
| 115 | Apr. 1, 1927 | 134 | 186 | \ | | . | .. | \ | \ | . | .... | Many | § | Left retropharyngeal one-third caseocalcareous. Mesenteric chain had 5 caseous nodules, 7 to 20 mm, and 1 colic node, caseocalcareous, 18 x 15 mm. |
| 116 | Apr. 13, 1927 | 117 | 323 | ..... | ..... | ..... | ..... | / | ..... | ..... | ..... | Few | + | 14 caseous nodules in mesenteric chain, 2 to 12 mm in size. |
| 117 | Apr. 16, 1927 | 116 | 339 | | . | | ... | . | / | .. | .. | Many | + | 3 caseocalcareous nodules, from 4 to 7 mm, in ileocecal lymph nodes. |
| 118 | May 1, 1927 | 104 | 360 | . | . | . | . | × | × | .. | .... | Many | + | Caseous and caseocalcareous lesions, 2 to 25 mm, scattered through the mesenteric and ileocecal lymph nodes. |
| 119 | May 29, 1927 | 76 | 186 | × | × | × | \ | × | .. | .... | .. | Many | + | Well-marked caseation in right lung, extensive caseation of retropharyngeal, and thoracic lymph nodes and caseous or caseocalcareous mesenteric lesions. |
| 120 | May 31, 1927 | 74 | 360 | .. | / | / | .. | × | × | ... | ... | Few | + | 1, 10-mm caseocalcareous nodule in bronchial, 1, 3-mm in post mediastinal and extensive caseocalcification of mesenteric and ileocecal nodes. |
| 121 | June 14, 1927 | 60 | 396 | / | .. | | .. | × | .. | . | .. | Many | + | 3, 3 to 4-mm caseocalcareous nodules in retropharyngeals and extensive caseous and slightly calcareous mesenteric lesions. |

TABLE 7A—(*Concluded*)

| No. | Born | Intervals in days | | | General condition on slaughter | Tuberculous lesions† | | | | | | | Acid-fast bacilli found in smears§ | Guinea-pig tests | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to vaccination‡ | Vaccination to first infecting feeding | First infecting feeding to autopsy | | Cervical | Bronchial | Mediastinal | Mesenteric | Cecal and colic | Gastro-hepatic | Other lesions | | | |
| 22 | June 13, 1927 | 7 | 54 | 396 | Fair | / | ..... | ..... | / | | . | .. | Few | + | 11 caseocalcareous nodules, 2 to 4 mm, scattered in mesenteric chain, one 2-mm nodule in retropharyngeal and a nodular connective tissue thickening in dewlap, 40 x 30 x 10 mm. |
| 23 | June 14, 1927 | 7 | 53 | 380 | Good | ..... | ..... | ..... | ..... | ..... | ... | ... .. | None | − | No lesions of tuberculosis. The dewlap contained a 50-mm thin-walled abscess of soft, creamy, odorless pus. No acid-fast bacilli found. |
| 24 | July 3, 1927 | 6 | 35 | 360 | Good | .... | .... | ... | / | / | ... | ... | Few | + | 15 caseous or caseocalcareous nodules, 3 to 12 mm, scattered through the mesenteric and cecal nodes. No dewlap lesions. |
| 25 | July 14, 1927 | 6 | 24 | 28 | See remarks | ..... | ..... | ..... | ..... | ..... | ..... | ..... | None | − | Died of acute gastritis too soon after infection to justify consideration. No tuberculous lesions found. |
| 26 | July 11, 1927 | 9 | 24 | 137 | Good | / | / | \ | / | .... | ... | .... | Few | + | Each retropharyngeal had a 5-mm necrotic area. Bronchials had 16, 0.5 to 5-mm caseous areas. 2 mediastinals had well-marked tuberculosis. 4, 4 to 10-mm mesenteric lesions. A 17-mm cold abscess in the dewlap. |
| 27 | July 30, 1927 | 6 | 8 | 382 | Fair | \ | .... | ..... | \ | / | ... | .. . | Few | + | Right postpharyngeal enlarged 2× and one-fifth caseocalcareous. 9 caseocalcareous nodules, 4 to 15 mm, scattered through the mesenteric and colic nodes. Dewlap lesion, 20 mm. |
| 28 | July 30, 1927 | 6 | 8 | 389 | Fair | \ | . | .... | / | / | / | / | Few | + | Retropharyngeal caseation, 18 x 15 x 12 mm. Slight lesions in liver and gastrohepatics. 14 caseocalcareous nodules, 2 to 12 mm, in mesenteric and cecal nodes. 5 hard, pea-sized nodules in dewlap. |
| 29 | Aug. 9, 1927 | 6 | −2 | 380 | Fair | / | / | / | / | ... | ..... | .... | Few | + | Vaccinated 2 days after first infecting feeding. Postpharyngeal, 1, 6-mm nodule. Left bronchial, 8, 3 to 5-mm nodules. Mediastinal, 3, 3-mm nodules. Mesenteric, 14, 2 to 10-mm nodules. Dewlap contained white connective tissue thickening, 10 x 5 x 20 mm. |

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves except animal No. 11, in which case the dose was administered in a long-necked bottle.

† Tuberculous lesion symbols: / slight, \ well marked, × extensive. For full explanation see footnote table 6.

‡ Figures in parentheses indicate the days to or from the second vaccination.

TABLE 7B—(*Concluded*)

| No. | Born | Intervals in days | | Tuberculous lesions† | | | | | | | | Acid-fast bacilli found in smears | Guinea-pig tests | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to first infecting feeding | First infecting feeding to autopsy | Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | Gastro-hepatic | Other lesions | | | |
| 122 | June 15, 1927 | 59 | 396 | ..... | ..... | / | ..... | × | × | ..... | ..... | Many | + | 2 caseocalcareous nodules, 4 to 5 mm in mediastinals. Extensive caseocalcareous lesions in the mesenteric and cecal lymph nodes. |
| 123 | June 22, 1927 | 52 | 380 | \ | ..... | ..... | ..... | / | / | ..... | ..... | Few | + | Caseocalcareous areas, 20 and 10 mm, in left postpharyngeal, 12 mm in right postpharyngeal, 4 mm in mediastinal, and 7 mm in a colic lymph node. |
| 124 | July 5, 1927 | 39 | 360 | × | ..... | / | ..... | × | \ | / | ..... | Few | + | A soft caseous lesion, 60 mm, in left postpharyngeal and 1, 10-mm in gastrohepatic. Extensive caseation in mesenteric and well-marked caseocalcareous lesions in ileo-cecal. |
| 125 | July 14, 1927 | 30 | 37 | ..... | ..... | ..... | ..... | \ | ..... | ..... | ..... | Many | + | Killed 37 days after infection because of the death of vaccinated calf No. 25, of which it was the control. Mesenteric lymph node was necrotic, 30 mm. |
| 126 | July 22, 1927 | 22 | 137 | × | ..... | ..... | ..... | / | / | ..... | ..... | Few | + | Retropharyngeal enlarged 2 x, caseous throughout. 8 caseocalcareous areas, 1 to 3 mm, in mesenteric and cecal nodes. |
| 127 | July 23, 1927 | 21 | 382 | \ | \ | \ | × | ..... | ..... | ..... | \ | Many | + | Lungs studded with caseocalcareous nodules. Caseous lesions in mediastinal and caseocalcareous lesions in retropharyngeal, bronchial, and pulmonal lymph nodes. |
| 128 | Aug. 6, 1927 | 7 | 389 | ..... | ..... | ..... | ..... | / | ..... | ..... | ..... | Many | + | Only 1 caseocalcareous 7-mm nodule in center of mesenteric chain of lymph nodes. |
| 129 | Aug. 7, 1927 | 6 | 380 | \ | ..... | / | ..... | \ | ..... | / | × | Many | + | Liver studded with 3 to 10-mm caseous nodules. Caseous lesions in mesenteric and mediastinal nodes and caseocalcareous in pharyngeal and gastrohepatic. |

* The infecting dose as described on table 5 was placed in a bucket and was voluntarily consumed by the calves, except animal No. 111, in which case the dose was administered in long-necked bottles.

† Tuberculous lesion symbols: / slight, \ well marked, × extensive. For full explanation see footnote, table 6.

‡ No smears made. § No test made.

## TABLE 8A

RESULTS FROM THIRTY FEEDINGS* OF TUBERCULOUS TISSUES TO CALVES VACCINATED IN THE DEWLAP WITH 100 MG BCG
(For controls, see table 8B)
Feeding dates: August 13 to September 9 as in tables 7A and 7B and also September 10, 12, 14, 16, 18, 19, 24, 28, 29, 30, and October 1–8, 11, and 12, 1927

| No. | Born (1927) | Intervals in days | | | General condition on slaughter | Tuberculous lesions† | | | | | Acid-fast bacilli found in smears‡ | Guinea-pig tests | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to vaccination | Vaccination to first infecting feeding | First infecting feeding to autopsy | | Cervical | Mesenteric | Cecal and colic | Gastro-hepatic | Spleen | | | |
| 30 | Mar. 30 | 8 (91)§ | 129 (46)§ | 186 | Good | ..... | \ | .. . | ..... | .... | Few | + | Caseation in 7 mesenteric nodes, 2 to 18 mm. Dewlap had 60-mm connective tissue thickening. |
| 31 | Apr. 4 | 5 | 124 | 323 | Good | .... | / | .... | ..... | .... | Few | + | 2 calcareous 5-mm nodules in mesenteric lymph nodes. Dewlap abscess, 40 mm, contained acid-fast bacilli. (See fig. 11.) |
| 32 | Apr. 13 | 9 (76) | 113 (46) | 331 | Fair | . ..... | / | / | ..... | \ | Few | + | Caseous nodule in spleen, 18 x 12 x 7 mm. Guinea pigs tuberculous from this spleen lesion. 32 caseocalcareous nodules, 2 to 7 mm, scattered in mesenteric and cecal nodes. |
| 33 | Apr. 26 | 4 | 105 | 380 | Good | . .... | / | / | . ..... | . ..... | None | + | 12, 2 to 5-mm caseocalcareous nodules in mesenteric nodes, and 4, 2 to 4-mm in cecal nodes. Dewlap had slight thickening of firm white connective tissue. |
| 34 | May 5 | 8 | 79 | 323 | Good | . | .... | ... . | ..... | .... | None | − | No lesions except 50-mm cold abscess in dewlap, containing yellow, soft, odorless pus and a few acid-fast bacilli. |
| 35 | May 30 | 6 | 70 | 339 | Good | \ | \ | \ | ..... | ... | Few | + | Soft caseous lesions in both retropharyngeals. 12 caseous areas in ileocecal nodes and 16 in mesenteric chain, ranging from 2 to 20 mm. No dewlap lesion. |
| 36 | June 1 | 3 (27) | 70 (46) | 382 | Good | / | / | / | / | ... | None | + | Right retropharyngeal and 1 gastrohepatic each had 1, 4-mm caseous nodule. 31 calcareocaseous areas, 5 to 10 mm, in mesenteric or colic nodes. Abscess in dewlap, 40 mm, contained acid-fast bacilli. |
| 37 | June 5 | 4 | 64 | 389 | Fair | / | / | .... | ..... | ..... | None | + | 1, 4-mm caseous nodule in right retropharyngeal and 15 caseocalcareous nodules, 3 to 8 mm, in mesenteric chain. Dewlap small abscess. |
| 38 | June 10 | 10 | 54 | 192 | Good | . . | / | / | ..... | .... | Few | + | 13 caseocalcareous nodules, 1 to 10 mm, in mesenteric chain and 3, 1-mm caseous points in an ileocecal node. Small caseous lesions in dewlap contained acid-fast bacilli. (See figs. 11 and 12.) |
| 39 | June 14 | 6 | 54 | 331 | Poor | ..... | × | ..... | ..... | ..... | Few | + | 20 soft caseous areas, 7 to 30 mm, in mesenteric chain. No dewlap abscess. |

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves.
† Tuberculous lesion symbols: / slight, \ well marked, × extensive. For further description see footnote, table 6.
‡ Does not include smears from the dewlap lesions.
§ Figures in parentheses indicate the days to or from second vaccination.

## TABLE 8B

RESULTS FROM THIRTY FEEDINGS* OF TUBERCULOUS TISSUE IN CONTROL CALVES NOT VACCINATED

Feeding dates: August 13 to October 12, 1927, as in table 8A

| No. | Born (1927) | Interval in days | | Tuberculous lesions† | | | | | | | Acid-fast bacilli in smears | Guinea-pig tests | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to first infecting feeding | First infecting feeding to autopsy | Cervical | Bronchial | Mediastinal | Mesenteric | Cecal and colic | Gastro-hepatic | Other lesions | | | |
| 130 | Apr. 1 | 107 | 186 | ... | / | . | × | \ | ... | / | Many | + | About one-half the mesenteric and ileocecal lymph nodes were caseocalcareous. Right bronchial and right prescapular nodes each had a small caseous lesion. |
| 131 | Apr. 14 | 121 | 323 | . . | . . . | . | \ | .. | . . . | . . | Many | + | 12 caseocalcareous and 5 calcareous nodules, 3 to 20 mm in size, scattered through the mesenteric chain. |
| 132 | Apr. 16 | 119 | 331 | . | ... | / | \ | / | | . . . | Many | + | Mediastinal 2-mm caseous nodule. Caseocalcareous mass 25 mm in mesenteric node, also 1, 4-mm. 4, 7 to 12-mm caseocalcareous nodules in cecal nodes. |
| 133 | Apr. 25 | 110 | 380 | . | . | .. | × | × | .... | . | Many | + | All mesenteric and about one-half the cecal and colic nodes enlarged and caseocalcareous or completely calcareous. |
| 134 | May 26 | 79 | 323 | .. | . | . . . | × | . | . . | × | Many | + | Extensive tuberculous serositis of serous surfaces in thorax and abdomen. Extensive caseocalcification in lower part of mesenteric chain. |
| 135 | May 31 | 74 | 339 | / | . . | . | × | / | .... | . | Many | + | 2, 4-mm caseous areas in right postpharyngeal. 2 caseocalcareous areas, each 30 mm, in ileocecal and 7, 2 to 4-mm in mesenteric chain. |
| 136 | May 31 | 74 | 382 | ..... | .... | . | \ | / | . . | | Many | + | 1, 4-mm caseocalcareous nodule in a cecal node and 10 such lesions in mesenteric chain, 4 to 15 mm. |
| 137 | June 7 | 67 | 389 | \ | .. | .... | / | \ | ... | .... | Few | + | A 15-mm caseocalcareous mass and several smaller nodules in each retropharyngeal node. 4, 7 to 10-mm caseocalcareous masses in mesenteric chain and 3, 4 to 15-mm in cecal node. |
| 138 | June 8 | 66 | 192 | \ | ..... | / | / | / | / | / | Many | + | Retropharyngeals caseocalcareous. Anterior and central mediastinals had several 2 to 4-mm caseous masses. 10 mesenteric and 1 colic node caseocalcareous. 1 gastrohepatic had a 4-mm caseous nodule and in a Peyer's patch was an 8-mm caseous area containing acid-fast bacilli. The right prescapular node had a 7-mm caseous nodule. (See figs. 13 and 14.) |
| 139 | June 12 | 62 | 331 | × | .... | / | × | × | ..... | .... | Many | + | Posterior mediastinal had a 4-mm caseocalcareous nodule. The colic and ileocecal nodes, and nodes in lower two-thirds of mesenteric chain were nearly all caseocalcareous. Both retropharyngeal nodes were enlarged and caseocalcareous. |

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves.

† Tuberculous lesion symbols: / slight, \ well marked, × extensive; for further details see footnote, table 6.

## TABLE 9A

RESULTS FROM THIRTY-SIX FEEDINGS* OF TUBERCULOUS TISSUE TO CALVES VACCINATED IN THE DEWLAP WITH 100 MG BCG

(For controls, see table 9*B*)

Feeding dates:* August 13 to October 12, 1927, as in tables 8*A* and 8*B*, and also October 14, 15, 16, 17, 19, and 22, 1927

| No. | Born | Intervals in days | | | General condition on slaughter | Tuberculous lesions§ | | | | | | | Acid-fast bacilli found in smears‡ | Guinea-pig tests | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to vaccination | Vaccination to first infecting feeding | First infecting feeding to autopsy | | Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | Other lesions | | | |
| | **1926** | | | | | | | | | | | | | | |
| 40 | May 16 | 2 (271)† | 451 (182)† | 132 | Good | / | ..... | ..... | ..... | / | / | ..... | None | + | Right retropharyngeal 4, 2 to 4-mm caseous nodules. Left retropharyngeal, 2, 2-mm nodules. In mesenteric nodes, 2, 2-mm necrotic masses. In a cecal node, a 4-mm necrotic mass. Cold abscess in dewlap contained acid-fast bacilli. |
| | **1927** | | | | | | | | | | | | | | |
| 41 | June 20 | 8 (56) | 46 (−2) | 397 | Poor | \ | ..... | ..... | ..... | / | / | ..... | + | + | Right retropharyngeal, a 35-mm caseous mass, 2, 4 to 5-mm caseocalcareous nodules in mesenteric and 1, 7-mm in ileocecal node. |
| 42 | July 4 | 5 (42) | 35 (−2) | 140 | Good | / | ..... | ..... | ..... | / | ..... | / | + | + | 1, 1-mm and 1, 5-mm necrotic masses in retropharyngeals. 4 mesenterics have sprinkling of pin point to 1-mm caseous foci. In Peyer's patches of ileum were 3 groups, numbering, respectively, 4, 2, and 7 hard, yellowish-gray, 2-mm nodules, containing acid-fast bacilli virulent for guinea pigs. Dewlap had connective tissue thickening, containing acid-fast bacilli. |
| 43 | July 12 | 8 | 24 | 383 | Good | / | / | / | ..... | / | ..... | / | None | + | Caseocalcareous nodules as follows: postpharyngeal, 3, 4 to 5-mm; left bronchial, 4, 3-mm; median mediastinal, 8, 1 to 5-mm; posterior mediastinal, 3, 1 to 5-mm; pulmonal, 7, 2 to 3-mm; mesenteric, 7, 1 to 4-mm. Dewlap abscess, 70-mm fluid pus containing acid-fast bacilli. |
| 44 | Aug. 5 | 9 | −1 | 215 | Poor | / | ..... | ..... | ..... | / | / | ..... | + | + | Emaciated. Musculature edematous. Caseous nodules in right retropharyngeal 6, 2 to 3-mm; left retropharyngeal, 5, 3 to 5-mm; left submaxillary, 1, 5-mm; mesenteric, 7, 3 to 8-mm. In the ileocecal nodes were 11, 1 to 10-mm, caseocalcareous nodules. Dewlap abscess, 40-mm caseation with acid-fast bacilli. |

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves.

† Figures in parentheses indicate the days to or from second vaccination.

‡ This column does not include smears from dewlap lesions.

## TABLE 9B

RESULTS FROM THIRTY-SIX FEEDINGS* OF TUBERCULOUS TISSUE TO CONTROL CALVES, NOT VACCINATED

Feeding dates: August 13 to October 12, 1927, as in tables 8*A* and 8*B*, and also October 14, 15, 16, 17, 19, and 22, 1927

| No. | Born | Interval in days: Birth to first infecting feeding | Interval in days: First infecting feeding to autopsy | General condition on slaughter | Tuberculous lesions†: Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | Gastro-hepatic | Other lesions | Acid-fast bacilli in smears | Guinea-pig tests | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1926 | | | | | | | | | | | | | | |
| 140 | Oct. 14 | 303 | 132 | Good | × | ..... | ..... | ..... | × | ..... | ..... | ..... | + | + | Both retropharyngeals enlarged 2½ x, and caseous throughout. 4 mesenteric nodes enlarged to 40 mm, studied with caseous points. Also, 3 other mesenteric nodes, each containing a 5 to 10-mm caseous mass. |
| | 1927 | | | | | | | | | | | | | | |
| 141 | June 13 | 61 | 397 | Poor | / | / | / | \ | × | ..... | × | \ | + | + | Gastrohepatics enlarged 3 x and caseocalcareous throughout. 23, 10 to 15-mm tubercles in liver. 8-mm caseocalcareous nodule in submaxillary. In right retropharyngeal 2, 8-mm; in left bronchial, 1, 20-mm and 5, 4 to 7-mm; in right bronchial, 2, 8-mm; in mediastinals, 8, 4 to 7-mm; in lung, 1, 5-mm and the mesenteric chain was two-thirds caseocalcareous. |
| 142 | July 13 | 31 | 140 | Fair | × | ..... | ..... | ..... | / | ..... | ..... | / | + | + | Left retropharyngeal enlarged 3 x; studded with 1 to 10-mm caseous nodules. A 2-mm caseous nodule in intestinal wall, and the mesenteric node draining it had a 4-mm caseous nodule. In a Peyer's patch, 5 ft. from the pyloric end of intestine was a 20-mm square area studded with 14 yellow-white nodules, each about 1 mm, and acid-fasts were present in smears. The mesenteric node draining the Peyer's patch contained a 25-mm caseous mass. |
| 143 | Aug. 5 | 8 | 383 | Fair | ..... | ..... | ..... | ..... | \ | \ | ..... | ..... | + | + | A colic node had 1, 18-mm caseocalcareous mass, and 2 mesenteric nodes had 2, 15 to 18-mm caseous lesions. |
| 144 | Aug. 7 | 6 | 215 | Fair | \ | .. | \ | ..... | \ | / | ..... | / | + | + | Caseocalcareous lesions as follows: Left retropharyngeal one-third involved; mediastinal, 1, 7-mm and 1, 15-mm; mesenteric chain, 1, 30-mm, 4, 2 to 8-mm; ileocecal, 1, 2-mm nodule; pulmonal lymph node had a 4-mm nodule. |

* The infecting dose as described in table 5 was placed in a bucket and was voluntarily consumed by the calves.

† Tuberculous lesion symbols: / slight, \ well marked, × extensive. For further description see footnote, table 6.

caseous or caseocalcareous lesions and the other calf had slight, but widely distributed lymph-node lesions. A literal and strict interpretation of the federal meat-inspection regulations would have resulted in the condemnation of all 4 carcasses.

Calves 11 to 24 differed from Nos. 26 to 29 in two respects, i.e., in the length of time after vaccination that they were infected and in their ages, the latter group being younger. A comparison of tables 7*A* and 7*B* shows that age was probably not a significant factor since well-marked or extensive lesions occurred in the nonvaccinated calves infected at ages from 6 to 199 days. The comparison also shows that, in general, the tuberculous lesions were more extensive in the control than in the vaccinated calves. It would seem, therefore, that the subcutaneous injection of BCG resulted, after 4 or 5 weeks, in the development of a certain resistance to tuberculosis. However, it was definitely shown that this resistance was not usually sufficient to prevent the penetration of the tissues of the alimentary tract by virulent tubercle bacilli and the production of caseous lesions in the mesenteric or cervical lymph nodes.

*Discussion of the Results of Thirty Infecting Feedings, as Given in Tables 8A and 8B.*—It will be noted from the table 8*A* that 9 of the 10 vaccinated calves had caseous or caseocalcareous lesions. In all but 1 of these, the visible tissue involvement was confined to the lymph nodes draining the pharyngeal or intestinal tracts. The exception was No. 32, in which was found a single tuberculous caseous nodule in the spleen, as well as 32 small caseocalcareous areas scattered through the lymph nodes draining the intestines. All 10 of the controls had well-marked or extensive caseous or caseocalcareous tuberculous lesions in 2 or more groups of lymph nodes.

On comparing the lesions in the vaccinated calves with their respective controls killed on the same date, it was observed that in one instance the lesions in the vaccinated calf (No. 36) were more widely distributed than in its control (No. 136) but not so extensive. The tissue involvment in calves 32 and 35 was about equal to that involved in their controls, 132 and 135. In the other 7 vaccinated calves, the lesions involved less tissue than in their respective controls. The results in tables 8*A* and 8*B* support the observations outlined in tables 7*A* and 7*B*, in that it is definitely shown that the vaccination with BCG did not prevent the penetration of the tissues of the alimentary tract by virulent tubercle bacilli and the production of caseous and caseocalcareous lesions in the adjacent lymph nodes. In general, however, the tuberculous lesions were more extensive in the control than in the vaccinated calves.

*Discussion of the Results of Thirty-six Infecting Feedings, as Given in Tables 9A and 9B.*—The 10 animals in this group had received 30 infecting feedings with the 20 calves listed in tables 8*A* and 8*B*, and it was decided to note what would result from the feeding of 6 more very massive doses of bovine tuberculosis lesions suspended in milk. On completing the autopsies, it was noted that, in general, no very marked differences existed, either in the extent or distribution of the lesions, which could be attributed to the additional infecting feedings.

All of the vaccinated calves showed widely distributed caseous or caseocalcareous tuberculous lesions in the lymph nodes. These lesions were small, ranging from a pin point to 10 mm. One retropharyngeal lesion, 35 mm in diameter, was found in calf 41, and small tuberculous lesions were also demonstrated in Peyer's patches of calf 42.

All of the control calves had widely distributed well-marked or extensive caseous or caseocalcareous tuberculous lesions of the lymph nodes. Pulmonary and hepatic tuberculosis existed in calf 141 and Peyer's patch tuberculosis in calf 142.

In general, the extent of the lesions in the vaccinated animals was less than in the controls and these findings substantiated the similar observations in the previous infection trials at this Station.

A comparison of the lesions in the calves which were revaccinated at about the beginning of the infecting feeding (Nos. 15, 20, 41, and 42) with the autopsy findings in other vaccinated calves revealed no significant difference. Therefore it would appear that the subcutaneous revaccination of calves during a time when they are exposed to infection will probably not result in any appreciable difference in their lesions.

It should be noted that calf 44, at 9 days of age, was vaccinated for the first and only time on the day after starting the infecting feedings. This calf was unthrifty and was slaughtered when 224 days of age or 215 days after the first infecting feeding. The animal was emaciated and its musculature was edematous. In spite of this, the only tuberculous lesions found were slight caseations of the cervical and abdominal lymph nodes. A guinea pig inoculated from portions of these lesions developed tuberculosis. Another guinea pig inoculated from portions of other tissues remained normal.

*Discussion of the Results of Feeding Milk from Tuberculous Udders, as Given in Tables 10A and 10B.*—The 4 calves, Nos. 45, 46, 145, and 146, received feedings daily for 63 days from the tuberculous udder of cow No. 600 (see fig. 9). This cow was milked twice daily,

## TABLE 10A

RESULTS FROM FEEDING* MILK FROM TUBERCULOUS UDDERS TO CALVES VACCINATED IN THE DEWLAP WITH 100 MG BCG

| No. | Born (1927) | Intervals in days | | | General condition on slaughter | Tuberculous lesions† | | | | | | Acid-fast bacilli found in smears‡ | Guinea-pig inoculation | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Birth to vaccination | Vaccination to infection | Infection to slaughter | | Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | | | |
| 45 | Sept. 11 | 6 | 77 | 103 | Fair | ..... | ..... | ..... | ..... | / | \ | None | + | Protected from tuberculous infection until the 83rd day of life. Then fed for 63 days on milk from cow 600 (see page 352). Butchered at 186 days of age. A lymph node of the mesenteric chain contained 6 caseous foci, pin point to 1 mm in size and a cecal node contained a 20-mm caseous mass. The dewlap connective tissue was thickened 20 mm and contained acid-fast bacilli. |
| 46 | Sept. 14 | 3 | 77 | 227 | Good | ..... | ..... | ..... | ..... | ..... | ..... | None | − | Protected from tuberculous infection until the 80th day of life. Then fed on milk from cow 600 (see page 352) for 63 days. Butchered at 307 days of age. No lesions resembling tuberculosis were found. Guinea pigs injected with cervical, thoracic, and abdominal lymph nodes remained normal. The dewlap contained a 70-mm thickening, but no acid-fast bacilli were found in it. |
| 47 | Sept. 12 | 5 | 13 | 94 | Fair | / | \ | \ | / | \ | × | Many | + | Born and reared until the 18th day of life in a tuberculosis-free herd. Then fed daily for 86 days, Sept. 30 to Dec. 25, 1927, on the milk from cow 2171 (see page 352). Butchered at 112 days of age. 4, 2 to 8-mm necrotic foci in retropharyngeals. One-third the volume of the bronchial and mediastinal nodes was caseous. 2, 2 to 5-mm nodules in the lung. All the cecal nodes were enlarged and caseous. Other portions of the mesenteric chain had 3 or 4, 1 to 2-mm necrotic points in many lymph nodes. A dewlap abscess, 37 mm, contained acid-fast bacilli. |
| 48 | Sept. 13 | 4 | 13 | 246 | Died, see remarks | / | ..... | ..... | ..... | ..... | ..... | Few | + | Same age, history, and period of infection as calf 47. Found dead in field at 263 days of age. Cause of death not known. Fair general condition of carcass. Left retropharyngeal had 9, 2-mm millet-like nodules grouped in a volume of less than 1 cc and the right retropharyngeal contained 13 such nodules in a group measuring about 1 cc. A 40-mm cyst in the dewlap contained acid-fast bacilli. |

* The feedings were either by direct nursing of cow, or else by pail feeding.

† Tuberculous lesion symbols: / slight, \ well marked, × extensive. For further description see footnote, table 6.

‡ Does not include smears from the dewlap lesions.

TABLE 10B

RESULTS FROM FEEDING* MILK FROM TUBERCULOUS UDDERS TO CONTROL CALVES NOT VACCINATED

| No. | Born (1927) | Purpose | Intervals in days | | General condition on slaughter | Tuberculous lesions† | | | | | | | | | Acid-fast bacilli found in smears | Guinea-pig inoculation | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Birth to infection | Infection to autopsy | | Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | Gastro-hepatic | Liver | Other lesions | | | |
| 145 | Sept. 14 | Control on 45 and 46 | 80 | 103 | Fair | \ | .. . | / | ..... | / | .. .. | ..... | ..... | ..... | Many | + | Protected from infection to 80th day of life. Then fed for 63 days on milk from cow 600. Butchered at 183 days of age. Left retropharyngeal studded with caseous masses. Posterior mediastinal had a 4-mm caseous focus. One mesenteric had a 4-mm caseous focus. |
| 146 | Sept. 16 | Control on 45 and 46 | 78 | 227 | Good | / | .. | .. . | ..... | \ | \ | ..... | ..... | ..... | Many | + | Protected from infection to 78th day of life. Then fed for 63 days on milk from cow 600. Butchered at 305 days of age. Parotid lymph node had a 4-mm caseous focus. In the mesenteric lymph nodes were caseocalcareous masses as follows: 3, 4-mm; 1, 10-mm; 1, 25-mm, and in the ileocecal 1, 25-mm. |
| 147 | Sept. 12 | Control on 47 and 48 | 18 | 62 | Died, see remarks | × | × | × | × | × | × | × | × | × | Many | + | Protected from infection to 18th day of life. Then fed until death (62 days) on milk from cow 2171. Died of tuberculosis at 80 days of age. Generalized pulmonary and lymphatic tuberculosis. |
| 148 | Sept. 14 | Control on 47 and 48 | 16 | 162 | Died, see remarks | × | × | × | × | × | × | ..... | ..... | × | Many | + | Protected from infection to 17th day of life. Then fed for 86 days on milk from cow 2171. Died of tuberculosis at 179 days of age. Generalized pulmonary and lymphatic tuberculosis. Lesions were caseocalcareous. |

* The feedings were either by direct nursing of cow, or else by pail feeding.

† Tuberculous lesion symbols: / slight, \ well marked, × extensive. For further description see footnote, table 6.

and the mixed secretion from all 4 teats was given to the 4 calves. An effort was made to give each calf approximately the same amount of milk. Acid-fast bacilli were demonstrated in smears from the left front quarter of the udder of cow No. 600 on several occasions, and, on autopsy, the parenchyma of the right rear and left front quarters was found to contain massive tuberculous lesions. This cow also had extensive pulmonary tuberculosis, and the calves were confined in an adjacent pen where she could reach over and lick them. In spite of the obviously massive infection to which the 4 calves were thus exposed, none of them showed lesions on autopsy which were extensive according to the classification used in these trials. In one of the vaccinated calves (No. 46), no lesions were found, and guinea pigs inoculated with bits of tissue from various lymph nodes remained normal. The other vaccinated calf had well-marked caseation of certain lymph nodes draining the intestine, as indicated in the table. The lesions in the 2 controls were more pronounced than in the vaccinated calf which showed lesions.

Cow No. 2171 (see fig. 10), from whose udder infecting feedings were given to calves Nos. 47, 48, 147, and 148, had been artificially infected on August 2 by thrusting a needle 2 inches into the glandular tissue of the right front and rear quarters and injecting spleen and lymph-node pulp from a guinea pig infected with bovine tuberculous lesions. As a result, the milk of these quarters was found to be virulent for guinea pigs on September 8 and 29, October 19, and November 5. Acid-fast bacilli were seen in smears of the milk of the 2 right quarters at various times between September 8 and November 25, 1927. On autopsy of cow No. 2171 on November 30, 1927, a tuberculous, necrotic focus was found in the parenchyma of each of the right quarters. Each focus was about 10 cm in diameter. No other lesions of tuberculosis were found in this cow.[9]

Calf No. 48 died in the pasture from unknown causes and was not found for 3 days. Post-mortem changes rendered the autopsy difficult; however, it is believed that no well-marked lesions of tuberculosis were present. Guinea pigs, inoculated from a postpharyngeal lesion in this calf, became tuberculous.

It is noteworthy that calves 47 and 48 were subjected to the infecting feedings only 13 days after vaccination when it might be supposed that the full immunizing effect of the vaccination had not yet developed.

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[9] Such limited localization of lesions resulting from inoculation of tuberculous material into the udder is unusual according to Cobbett.(26)

The control calves, were of about the same age and size as the vaccinated. They were reared and fed together and were comparable in every way except for the vaccination in the dewlap.

It will be noted that an evident difference exists in the susceptibility of the vaccinated calves listed in table 10*A* and the unvaccinated calves listed in table 10*B*. Two out of 4 controls died of tuberculosis, while all of the vaccinated animals remained in good or fair condition up to the time of death.

These findings are of significance when added to the data in previous tables because they substantiate the results already obtained—that the subcutaneous vaccination will not prevent the penetration of the tissues by virulent tubercle bacilli but the lesions remain localized in the lymph nodes draining the points of entry of the bacilli, while in nonvaccinated calves the result may be a generalized tuberculosis.

## INTRAVENOUS, ORAL, AND INTRADERMIC VACCINATION

It was decided to test the protective effect, if any, of vaccination of calves by various other methods besides the injection into the dewlap as recommended by Calmette and Guérin.[20] Accordingly, as indicated in table 11*A*, 3 calves were vaccinated intravenously, 6 were given 3 vaccinating feedings each, and 3 were injected intradermally. As in previous trials the vaccinated animals and an equal number of controls were carefully protected from tuberculous infection until the time they were subjected to infection by feeding tuberculous bovine tissues suspended in milk. A few days before the first infecting feeding the controls were given an intradermic tuberculin test, but no reactions occurred. The detailed preparation of the infecting material will be found in table 5, and the results are summarized in tables 11*A* and 11*B*.

*Discussion of Tables 11A and 11B.*—It will be noted that in each group 6 animals received only 1 infecting feeding, while the other 6 received 3 infecting feedings. A study of the controls in table 11*B* indicates that the 1 feeding on July 7 was as effective in producing extensive lesions as the 3 feedings on July 7, 9, and 11, since extensive lesions occurred in every one of the control calves which received the 1 feeding on July 7, while in 2 of the calves which received 3 feedings, the lesions were only well marked. This supports the data already presented in tables 6 to 9, showing that continued feeding of large doses of virulent tubercle bacilli has little apparent effect in increasing the severity or extent of the lesions produced.

## TABLE 11A

RESULTS FROM FEEDING TUBERCULOUS TISSUES TO CALVES VACCINATED INTRAVENOUSLY, BY MOUTH, OR INTRADERMALLY

| No. | Born (1928) | Vaccination with BCG | Number of infecting feedings* | Intervals in days | | General condition | Tuberculous lesions† | | | | | | Acid-fast bacilli found in smears‡ | Guinea-pig inoculations | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | First vaccination to first infection | First infection to autopsy | | Cervical | Bronchial | Mediastinal | Mesenteric | Cecal and colic | Other lesions | | | |
| | | Intravenously: | | | | | | | | | | | | | |
| 49 | Jan. 27 | 100 mg, Feb. 3 | 1 | 155 | 207 | Good | ..... | ..... | ..... | / | ..... | ..... | None | + | 2 mesenteric lymph nodes, each having 1, 2-mm caseous point. |
| | | By mouth: | | | | | | | | | | | | | |
| 50 | Jan. 5 | Jan. 16, 1 gm<br>Jan. 18, 1 gm<br>Jan. 20, 1 gm | 1 | 173 | 192 | Good | / | ..... | ..... | / | / | / | Few | + | 2, 2-mm caseous foci in retropharyngeal, 5, 1 to 2-mm in pulmonal, 31 caseocalcareous nodules 1 to 12 mm in mesenteric, and 3, 2 to 3-mm in ileocecal lymph nodes. |
| | | By mouth: | | | | | | | | | | | | | |
| 51 | Apr. 9 | Apr. 13, 3 gms<br>Apr. 15, 2 gms<br>Apr. 17, 2 gms | 1 | 85 | 186 | Fair | / | ..... | ..... | ..... | ..... | ..... | Few | + | Right retropharyngeal lymph node had 15 caseous foci 1 to 4 mm in size. |
| | | By mouth: | | | | | | | | | | | | | |
| 52 | Apr. 10 | Apr. 13, 3 gms<br>Apr. 15, 2 gms<br>Apr. 17, 2 gms | 1 | 85 | 186 | Fair | / | ..... | ..... | ..... | ..... | ..... | Few | + | 9 caseous points 1 to 4 mm in size in left, and 17 in right retropharyngeal lymph node. |
| | | Intradermally: | | | | | | | | | | | | | |
| 53 | Jan. 27 | 100 mg, Feb. 3 | 1 | 155 | 207 | Good | / | ..... | ..... | . | ... | ..... | Few | + | A 2-mm caseous nodule in right retropharyngeal lymph node. |
| | | Intradermally: | | | | | | | | | | | | | |
| 54 | Feb. 21 | 100 mg, Feb. 29 | 1 | 129 | 3 | Died | ..... | ..... | ..... | ..... | .. | ..... | None | − | Died of gastroenteritis too soon after infection to justify consideration. No tuberculous lesions were found. |

TABLE 11B

RESULTS FROM FEEDING* TUBERCULOUS TISSUES TO CONTROL CALVES KEPT WITH THE ANIMALS LISTED IN TABLE 11A

| No. | Born (1928) | Number of infecting feedings | Intervals in days | | General condition at autopsy | Tuberculous lesions† | | | | | | | | | Acid-fast bacilli found in smears | Guinea-pig inoculation | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Birth to first infecting feeding | First infecting feeding to autopsy | | Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | Gastro-hepatic | Liver | Other lesions | | | |
| 149 | Jan. 22 | 1 | 167 | 191 | Fair | × | / | / | .... | × | × | × | × | × | Many | + | Extensive caseous lesions in liver and in Peyer's patches of small intestines. Extensive caseous and caseocalcareous masses in gastrohepatic, mesenteric, and cervical, and slight caseation of bronchial and mediastinal. |
| 150 | Jan. 21 | 1 | 166 | 207 | Good | × | .. | / | .... | × | / | ..... | ..... | ..... | Few | + | Well-marked caseation of Peyer's patches and extensive soft caseation of mesenteric and right retropharyngeal, and small caseous foci in mediastinal and cecal lymph nodes. |
| 151 | June 4 | 1 | 33 | 186 | Fair | × | .... | .... | ..... | × | \ | ... | ... | .... | Many | + | Lower third of mesenteric chain extensively caseocalcareous. Massive caseocalcification in retropharyngeal. Scattered cecal, and colic node lesions. |
| 152 | June 15 | 1 | 22 | 186 | Fair | × | .... | / | ..... | / | / | ..... | ..... | ..... | Many | + | Extensive caseation of retropharyngeals and a few caseocalcareous foci in submaxillary, mediastinal, mesenteric, and cecal nodes. |
| 153 | Jan. 24 | 1 | 169 | 207 | Good | \ | / | / | ... | \ | / | × | × | / | Few | + | Numerous 2 to 4-mm nodules in liver. Extensive caseation of gastrohepatic; well-marked in the retropharyngeal and mesenteric and slight in the bronchial, mediastinal, pulmonal, and cecal nodes. |
| 154 | Feb. 25 | 1 | 132 | 191 | Fair | × | / | / | / | × | × | × | / | \ | Many | + | Well-marked Peyer's patch tuberculosis and extensive or slight lymphatic caseation as indicated in symbol columns of this table. |

TABLE 11A—(*Concluded*)

| No. | Born (1928) | Vaccination with BCG | Number of infecting feedings* | Intervals in days: First vaccination to first infection | Intervals in days: First infection to autopsy | General condition | Tuberculous lesions†: Cervical | Bronchial | Mediastinal | Mesenteric | Cecal and colic | Other lesions | Acid-fast bacilli found in smears‡ | Guinea-pig inoculations | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 55 | Jan. 31 | Intravenously: 100 mg, Feb. 3 | 3 | 155 | 192 | Fair | . | ... | ..... | / | / | ..... | Few | + | 17 caseocalcareous nodules, 1 to 5 mm, in mesenterics and 2, 2-mm foci in ileocecal lymph nodes. |
| 56 | Feb. 3 | By mouth: Feb. 8, 1 gm; Feb. 10, 1 gm; Feb. 12, 1 gm | 3 | 150 | 160 | Died | / | ..... | ..... | ..... | ..... | ..... | Few | + | Cause of death unknown. Left retropharyngeal had 1, 3-mm caseous nodule, right had 4, 1 to 3-mm necrotic foci. |
| 57 | Feb. 26 | By mouth: Feb. 27, 1 gm; Feb. 29, 1 gm; Mar. 2, 1 gm | 3 | 131 | 207 | Good | \ | / | / | / | .... | .... | Few | + | Well-marked caseation of both retropharyngeals; right bronchial had 2, 2-mm, anterior mediastinal 7, 2 to 3-mm, and mesenteric chain of lymph nodes 32, 2 to 4-mm caseous nodules. |
| 58 | Apr. 13 | By mouth: Apr. 13, 3 gms; Apr. 15, 2 gms; Apr. 17, 2 gms | 3 | 85 | 186 | Fair | ..... | ..... | ..... | ..... | ..... | ..... | None | + | No visible tuberculous lesions. Guinea pig injected with cervical lymph node tissues became tuberculous. |
| 59 | Feb. 1 | Intradermally: 100 mg, Feb. 3 | 3 | 155 | 191 | Fair | \ | ..... | / | ..... | ..... | ..... | Few | + | Well-marked caseation of both retropharyngeal and numerous 2 to 5-mm caseous foci in mesenteric lymph nodes. |
| 60 | June 8 | Intravenously: 100 mg, June 18 | 3 | 53 | 152 | Fair | ..... | ..... | ..... | ..... | ..... | ..... | None | + | No visible tuberculous lesions. Guinea pigs injected with cervical and with mesenteric lymph-node tissue became tuberculous. |

* The infecting feedings were given through a rubber tube. For details regarding dates and dosage see table 5.
† Tuberculous lesion symbols: / slight, \ well marked, × extensive. For details see footnote, table 6.
‡ Does not include smears from the dewlap lesions.

TABLE 11B—(*Concluded*)

| No. | Born (1928) | Number of infecting feedings | Intervals in days | | General condition at autopsy | Tuberculous lesions† | | | | | | | | | Acid-fast bacilli found in smears | Guinea-pig inoculation | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Birth to first infecting feeding | First infecting feeding to autopsy | | Cervical | Bronchial | Mediastinal | Lungs | Mesenteric | Cecal and colic | Gastro-hepatic | Liver | Other lesions | | | |
| 155 | Jan. 21 | 3 | 166 | 207 | Good | \ | / | / | . | \ | / | ... | ..... | / | None | + | Well-marked caseocalcification in retropharyngeal and mesenteric and slight in pulmonal, bronchial, mediastinal, and cecal nodes. |
| 156 | Feb. 5 | 3 | 152 | 160 | Good | × | / | / | ..... | × | × | / | ..... | \ | Few | + | Extensive caseocalcification of mesenteric, cecal, colic, retropharyngeal, and a few 2 to 3-mm nodules in bronchial, mediastinal, and gastrohepatic lymph nodes. Well-marked caseation in Peyer's patches. |
| 157 | Apr. 14 | 3 | 84 | 191 | Fair | × | ..... | ..... | ..... | / | / | ..... | ..... | / | Few | + | 24, 1 to 12-mm caseocalcareous nodules in mesenteric, 1, 3-mm in cecal, 1 4-mm in a Peyer's patch, and extensive caseocalcification of the retropharyngeal lymph nodes. |
| 158 | June 15 | 3 | 22 | 186 | Fair | × | ..... | / | ..... | \ | \ | / | ..... | ..... | Many | + | Extensive caseocalcification in retropharyngeal, and well-marked in mesenteric and ileocecal, and a few 2-mm foci in gastrohepatic and mediastinal. |
| 159 | Feb. 25 | 3 | 132 | 191 | Fair | ..... | ..... | / | ..... | × | \ | ..... | ..... | / | Many | + | Extensive caseocalcification in mesenteric chain. 1 caseous mass in a Peyer's patch, cecal nodes studded with 2 to 3-mm nodules. 1, 1-mm nodule in a mediastinal node. |
| 160 | June 5 | 3 | 66 | 152 | Fair | \ | ..... | ..... | ..... | \ | \ | ..... | ..... | / | None | + | 3, 15 to 22-mm caseocalcareous masses in retropharyngeal and 84 in mesenteric, cecal, and colic lymph nodes. |

* The infecting feedings were given through a rubber tube. For details regarding dates and dosage see table 5.

† Tuberculous lesion symbols: / slight, \ well-marked, × extensive. For details see footnote, table 6.

From table 11*A* it will be noted that the time elapsing between vaccination and infection ranged from 53 to 173 days. The lesions in the vaccinated calves were well marked in 2 cases (Nos. 57 and 59), slight but widely distributed in 1 (No. 50), slight and localized in 6 (Nos. 49, 51, 52, 53, 55, and 56) and in 2 (Nos. 58 and 60) no evidence of tuberculous infection could be found. No. 54 died of intercurrent disease and was not taken into consideration. In contrast, the controls all had well-marked or extensive widely distributed tuberculous lesions.

The number of calves used in the trials described in table 11*A* is not sufficient to justify final conclusions, but a comparison with the results in the controls (table 11*B*) supports the evidence obtained in the previous trials of BCG given subcutaneously. A resistance to subsequent infection is produced by the presence of the BCG organisms in the tissues; as a rule, however, this is not sufficient to prevent the penetration of the body by virulent tubercle bacilli and the production of caseation or caseo-calcification in the lymph nodes.

From the limited number of calves used (12), there is nothing to indicate that the administration of the BCG vaccine by mouth, intravenously, or intradermally had any more immunizing effect than when given subcutaneously in the dewlap.

## THE USE OF BCG IN A TUBERCULOUS HERD

On the first visit to the Williamson farm on Grisly Island in April, 1926, to begin the use of BCG, it was evident that extensive tuberculous infection existed. Several of the cows had enlarged retropharyngeal or prescapular lymph nodes, and a few were observed with symptoms of advanced pulmonary lesions. Guinea-pig inoculations from 2 of the animals proved the existence of cases of open tuberculosis in the herd. An intradermic tuberculin test, applied to all the cattle in the herd, showed 55 per cent positive reactors. The results of this test, grouped according to the ages of the cattle, are given in table 12.

The calves born on this dairy since 1920 had been permitted to nurse their dams for from 2 to 5 days, after which they were transferred to nurse cows or fed from pails on the mixed raw milk or skim milk of the entire herd.

In 1926 at the time the experiment was started, Calmette and Guérin had not retracted their published statement that it was unnecessary to take special sanitary precautions or to heat the milk from the tuberculous cows before feeding it to the calves. It was evident to

the writers that the vaccine would be more likely to be effective if the calves could be protected from infection for at least 30 days after vaccination. However, the owner desired to proceed with the trial on the basis of the published recommendations of Calmette and Guérin existing at that time, and it was decided to accede to his urgent request in the hope that the majority of the calves would escape infection during the first month of life and that by repeated vaccination over a period of several years, it would be possible to build up a resistant herd.

TABLE 12

TUBERCULIN TEST, H. D. WILLIAMSON DAIRY, APRIL, 1926

One-tenth cc of 10 per cent solution alcohol-precipitated tuberculin was injected into left subcaudal fold.

| Age | Number tested | Number positive reactions | Number indecisive reactions | Number negative |
|---|---|---|---|---|
| Less than one year | 27 | 4 | 0 | 23 |
| 1 to 2 years | 38 | 23 | 0 | 15 |
| 2 to 3 years | 43 | 18 | 0 | 25 |
| 3 to 4 years | 25 | 14 | 1 | 10 |
| 4 to 5 years | 20 | 13 | 0 | 7 |
| 5 to 6 years | 15 | 10 | 0 | 5 |
| 6 to 7 years | 19 | 14 | 2 | 3 |
| 7 to 8 years | 29 | 23 | 2 | 4 |
| 8 to 9 years | 1 | 1 | 0 | 0 |
| 10 to 15 years | 4 | 3 | 0 | 1 |
| 15 to 20 years | 2 | 0 | 0 | 2 |
| Total | 223 | 123 | 5 | 95 |

During 1926, 38 calves were vaccinated and 10 were left unvaccinated as controls. Since that time, few controls have been left, because the owner insisted on having all of the calves vaccinated in the hope that sufficient resistance would be created to prevent the development of any open cases of tuberculosis in the vaccinated animals and that eventually he could dispose of all unvaccinated cattle, cease vaccination and develop a nonreacting herd from the offspring of the vaccinated animals.

The vaccinating dose used in 1926 was 50 mg. Since that year in all cases the dose has been 100 mg injected into the dewlap, the first injection being given from 1 to 10 days after birth. The revaccinations have been made in 4 to 10 months following the initial injection and annually thereafter. The numbers of calves vaccinated each year are listed in table 13.

In accordance with the recommendations of Calmette, no tuberculin has been used on the vaccinated calves since 1926, with the exception

of 4 animals just before slaughter. The owner was advised that, if tested, they would probably react for a time as a result of the vaccination regardless of whether or not they developed tubercles other than those in the dewlap. (See the section "Hypersensitiveness in Vaccinated Calves," pp. 366.) He was also advised in 1927 that the most that could be expected from the vaccine was a resistance and that it would not prevent the penetration of the tissues by virulent tubercle bacilli. However, he decided to continue the vaccination of all the calves.

TABLE 13

CALVES VACCINATED AT H. D. WILLIAMSON DAIRY

| Year born | Number vaccinated | | | | |
|---|---|---|---|---|---|
| | First time | Second time | Third time | Fourth time | Fifth time |
| 1926 | 38 | 33 | 26 | 25 | 22 |
| 1927 | 58 | 51 | 50 | 43 | |
| 1927 | 6 | 6 | 6 | | |
| 1928 | 44 | 40 | | | |
| 1929 | 46 | | | | |
| Total | 192 | | | | |

The following year he had some reason to hope that the majority of them would escape infection during the first 30 days of life because 3 vaccinated calves, reared on the place for from 8 to 12 weeks, had been taken to Berkeley and no tuberculous lesions were found on slaughter after 9, 16, and 18 months, respectively. On the other hand, a nonvaccinated calf of this group was found to be tuberculous on slaughter.

Guinea-pig inoculations from the mixed milk of the herd on one occasions in 1927 resulted in producing tuberculosis.

In September, 1927, one of the 6-week-old calves which had been vaccinated 5 days after birth was observed with labored breathing and coughing. On slaughter at 2 months of age, extensive tuberculous lesions were found in the lungs and thoracic lymph nodes. A guinea pig inoculated from these lesions died of generalized tuberculosis, but a guinea pig inoculated from the vaccination abscess in the dewlap was normal throughout when slaughtered 2 months after inoculation. On investigation, it was found that the cow on which this calf had nursed had tuberculosis of the udder (see cow No. 600, page 352). A non-vaccinated control calf which had been nursing on this cow also developed pulmonary symptoms and on slaughter was found to have tuberculosis of the mesenteric lymph nodes and non-

tuberculous bronchopneumonia. With the previous exception, none of the vaccinated calves in this herd has apparent clinical tuberculosis.

In the winter of 1928, 2 of the nonvaccinated controls, born in 1926, were reported by the owner to have died of tuberculosis. Unfortunately, no tissues were saved and the University veterinarian was not notified.

During 1929 it has been possible to perform autopsies on 10 of the 3-year-old heifers, 5 of which had been repeatedly vaccinated and 5 of which were nonvaccinated controls. The results are given in tables 14*A* and 14*B*.

*Discussion of Tables 14A and 14B.*—The extent of exposure of the cattle in this field experiment to tuberculous infection cannot be as satisfactorily measured as the controlled infection trials at Berkeley and Davis. However, from 8 nonvaccinated animals available for tuberculin testing or slaughter, 5, or 62.5 per cent, developed tuberculosis. These 5 are listed in table 14*B*. One of them, No. 205, was killed because it was in an extremely poor and weak condition as a result of tuberculous infection. The other 4 were selected from 7 available nonvaccinated animals because they yielded strong positive reactions to the intradermal tuberculin test. The 3 animals negative to this test were not slaughtered, but were retained in the Williamson herd to serve as controls in future observations.

At the same time that the above 7 nonvaccinated heifers were tested with tuberculin by the intradermic method, 4 vaccinated heifers, of about the same age as the nonvaccinated controls, were selected at random from a group of 20, which, in 1926, had been vaccinated within 10 days of birth and which had been revaccinated 4 times since that year. The test on these 4 animals failed to show a degree of hypersensitiveness sufficient to cause a reaction definite enough to condemn any of the animals under the standards ordinarily used in routine testing. They were slaughtered for beef at the same time as the 4 controls.

None of the 5 vaccinated animals recorded in table 14*A*, showed any macroscopic evidence of tuberculosis. One, No. 236, did, however, carry live tubercle bacilli in one or more of its thoracic lymph nodes, since the guinea pigs injected with these apparently normal lymph nodes developed tuberculosis. The 4 heifers recorded in table 14*A* were selected at random from a vaccinated group in which all the animals were apparently in the same approximate state of health.

It will be noted that heifer 216 (table 14*A*) showed tuberculous lesions in the prescapular lymph node. The right prescapular node measured 80 x 40 x 30 mm and contained 6 caseous points, each 1 to

## TABLE 14A

### Heifers Vaccinated in the Dewlap with 100 Mg BCG and Maintained from Birth with a Tuberculous Herd

| No. | Born | Dates vaccinated | Remarks |
|---|---|---|---|
| 264 | Sept. 1, 1926 | Sept. 4, 1926<br>Mar. 25, 1927<br>Oct. 18, 1927<br>July 18, 1928<br>July 15, 1929 | Calved Feb., 1929. Drowned accidentally Aug. 14, 1929. No evidence of tuberculosis found, except three vaccination abscesses in the dewlap. Guinea pigs, inoculated from dewlap abscess and cervical, mesenteric, and thoracic lymph nodes, were negative for tuberculosis at 3 months. |
| 268 | Sept. 1, 1926 | Sept. 1, 1926<br>Mar. 25, 1927<br>Oct. 18, 1927<br>July 18, 1928<br>July 15, 1929 | Calved Feb., 1929. Negative to intradermic tuberculin test Aug. 29, 1929. Slaughtered for beef Sept. 4, 1929. No evidence of tuberculosis found, except eight 15-mm abscesses in the dewlap. Guinea pigs inoculated with cervical and thoracic lymph node tissue were negative for tuberculosis at 10 weeks. |
| 236 | June 29, 1926 | July 3, 1926<br>Mar. 25, 1927<br>Oct. 18, 1927<br>July 18, 1928<br>July 15, 1929 | Calved Mar., 1929. Negative to the intradermic tuberculin test Aug. 29, 1929. Slaughtered for beef Sept. 4, 1929. No evidence of tuberculosis found, except 3 abscesses in the dewlap, each 50 mm. Guinea pigs inoculated from the apparently normal thoracic lymph nodes were positive for tuberculosis at 10 weeks. |
| 216 | May 23, 1926 | June 3, 1926<br>Mar. 26, 1927<br>Oct. 18, 1927<br>July 18, 1928<br>July 15, 1929 | Calved March, 1929. Negative to the intradermic tuberculin test Aug. 29, 1929. Slaughtered for beef Sept. 4, 1929. No evidence of tuberculosis found, except in the dewlap and adjacent prescapular lymph nodes (see page 00). Guinea pigs inoculated from the cervical thoracic and prescapular lymph nodes, were negative for tuberculosis at 10 weeks. |
| 212 | May 11, 1926 | May 21, 1926<br>Mar. 21, 1927<br>Oct. 18, 1927<br>July 18, 1928<br>July 15, 1929 | Calved Mar. 19, 1929. Negative to the intradermic tuberculin test Aug. 29, 1929. Slaughtered for beef Sept. 4, 1929. No evidence of tuberculosis found, except 3 walnut-sized abscesses in the dewlap. Guinea pigs inoculated from the cervical thoracic and gastrohepatic lymph nodes, were negative for tuberculosis at 10 weeks. |

## TABLE 14B

### Nonvaccinated Heifers Serving as Controls for Heifers Listed in Table 14A

| No. | Born | Remarks |
|---|---|---|
| 205 | April, 1926 | Calved March 1, 1929, after which symptoms of generalized tuberculosis appeared. Killed April 21, 1929. Massive tuberculosis of organs and lymph nodes of the thoracic and abdominal cavities. Acid-fast bacilli were abundant in uterus and bronchial tubes. |
| 203 | April, 1926 | Slaughtered for beef, September 4, 1929. A mesenteric lymph node contained a calcareous nodule, 20 x 15 x 8 mm, and in another node near by was a 7-mm caseous area. Acid-fast bacilli were demonstrated and inoculated guinea pigs developed tuberculosis. |
| 225 | Jan., 1926 | Slaughtered for beef, September 4, 1929. The left retropharyngeal lymph node was enlarged 3 times normal size and about one-third of its area was thickly studded with caseocalcareous nodules, from 5 to 15 mm in size, typical of tuberculosis. |
| 219 | May, 1926 | Slaughtered September 4, 1929. Condemned as unfit for food on account of emaciation. Extensive cervical and thoracic tuberculosis. |
| 473 | April, 1926 | Slaughtered for beef, September 4, 1929. The left bronchial lymph node was enlarged 6 times normal size and contained a caseocalcareous lesion, 15 mm in size, in which acid-fast bacilli were demonstrated. |

2 mm in size. These were grouped in a space 10 x 7 x 5 mm. The left prescapular node measured 70 x 40 x 20 mm and contained 8 caseous points, ranging from 2 to 3 mm in diameter. These were grouped together near the surface of the node. Near by was a yellow caseous mass, 3 x 4 x 3 mm in size. Acid-fast bacilli were demonstrated in smears from the above lesions as well as in the pus of several small abscesses at the points of vaccination in the dewlap. It appeared that the BCG organisms injected into the dewlap had penetrated the adjacent lymph nodes. The fact that guinea pigs inoculated from the prescapular lymph nodes failed to develop tuberculosis supports the idea that the prescapular lesions were caused by the BCG organisms and not by ingested acid-fast bacilli.

The fact that none of the vaccinated animals (table 14*A*) showed any macroscopic lesions attributable to virulent tubercle bacilli, while all of their controls (table 14*B*) had definite lesions, strongly supports the claims of Calmette.

It has been customary to slaughter all male calves for veal at the age of 2 or 3 months. To date, about 200 have been so slaughtered by the superintendent of the ranch, who reports that no lesions resembling tuberculosis have been observed except in the previously mentioned cases. In addition, tissues from several of these calves were sent to Berkeley and portions were injected into guinea pigs with negative results.

For economic reasons the owner has wished to sell some of the vaccinated heifers. They have, however, been found unmarketable except for beef, because it is supposed that they will probably react if subjected to the tuberculin test.

Until there is an opportunity to make post-mortem studies on more of the heifers which have been on the farm since 1926, the only conclusions that the writers are able to draw from the use of BCG in this herd are as follows:

1. No apparent injury to the general health or milking efficiency of the heifers has been produced by the annual vaccination.

2. A comparison in 1929 of the vaccinated and nonvaccinated heifers born in 1926 shows that the vaccinated heifers are all in good condition, while some of the nonvaccinated have died of tuberculosis. Five vaccinated heifers born in 1926 were found apparently sound on autopsy in 1929, while an equal number of nonvaccinated controls were found to be tuberculous.

3. Since the vaccine causes animals to become temporarily reactors to tuberculin, the vaccinated heifers were rendered for a time unmarketable, except for beef.

4. The results already obtained in the Williamson herd, when considered in connection with the results of the controlled experiments at Berkeley, are a distinct addition to knowledge concerning immunity and resistance to tuberculosis. A continuation of the cooperation with Mr. Williamson has therefore been arranged.

## OBSERVATIONS OF THE EFFECT OF BCG ON CATTLE NOT EXPOSED TO TUBERCULOUS INFECTION

To determine if BCG cultures may cause any injury to cattle is of fundamental importance and a necessary prerequisite to extensive vaccination trials. Particularly, it is necessary to find out if extensive tuberculosis may result directly from the vaccination. It has been demonstrated by investigation in various countries, as well as by Calmette's group, that local tuberculous lesions are produced in the tissues of animals at the points where the living BCG bacilli lodge. The majority of reports concerning this indicate that these local lesions do not progress to an extent that is permanently detrimental to health and that if the animals are premitted to live, the lesions eventually heal. Consequently, the Commission of Bacteriologists of the Hygiene Section of the League of Nations[28] reported in 1928 that BCG constitutes a harmless vaccine for cattle. On the contrary, Watson[74] has reported that passages of BCG from calf to calf resulted, in one instance, in the development of virulence sufficient to cause generalized tuberculosis in guinea pigs inoculated from the calves.

To date, 27 head of cattle in the nontuberculous herd belonging to the University at Berkeley have been subjected to injections of BCG in various ways. Following is a list of these trials:

1. Eight aged cows were injected in the dewlap with BCG, dose 100 mg. On slaughter after 72 days, no tuberculous lesions were found except the usual cold abscesses at the point of vaccination.

2. Fifteen calves born in 1926 and 1927, were vaccinated in the dewlap from 1 to 10 days after birth, and have been revaccinated annually since that time. To date (November, 1929), no injurious effect has been noted from the vaccine other than the local abscess produced in the dewlap. In one of these animals (No. 1111) after the second vaccination on February 12, 1927, the dewlap lesion gradually developed to such a size that it became an objectionable blemish. In September, 1928, it consisted of multiple intercommunicating abscesses, forming 3 tumors having respective diameters of 150, 80 and 70 mm. The contents consisted of semi-solid caseocalcareous, necrotic tissue.

On microscopic examination, large numbers of acid-fast bacilli were found mixed with leucocytes and amorphous, necrotic debris. Cultures on serum egg and other media remained sterile. Four guinea pigs inoculated with the pus were killed after 6 months and found to be in excellent condition. No lesions of tuberculosis were found in them.

An attempt was made to reduce the size of the abscesses in calf No. 1111 by free incisions, drainage, and the liberal use of tincture of iodine, without much effect; but, after several injections of hexylresorcinol, the discharges ceased and healing resulted.

On December 19, 1928, the animal was revaccinated in the dewlap with 100 mg of BCG. In August, 1929, the 3 swellings in the dewlap measured respectively 110, 110, and 80 mm.

3. Three calves, aged 2 months each, received the relatively massive doses, 1 to 4 grams, distributed in smaller amounts at various points

TABLE 15

THE RESULTS OF INTRAVENOUS INJECTIONS OF BCG IN CATTLE AND SWINE

| No. | Sex and species | Age when injected | Dose of BCG, mg | Remarks |
|---|---|---|---|---|
| 82 | Steer | 5 months | 100 | Temperature remained normal. Butchered 30 days after injection of BCG. Tissues were apparently normal throughout on macroscopic examination except that on close scrutiny the lungs were seen to be studded throughout with glass-like nodules, pin point to 0.5 mm in size and occasionally a larger nodule (1 mm) was observed. A histological study showed the lungs to be thickly studded with foci containing acid-fast bacilli (figs. 15 and 16). Guinea pigs injected with lung and lymphatic tissue remained normal. |
| 94 | Steer | 2 months | 50 | Temperature irregular for 4 weeks; otherwise the animal appeared normal. Slaughtered 4 months after injection. Tissues normal throughout. Lungs normal histologically. Guinea pigs injected with lung and lymphatic tissue remained normal. |
| 1130 | Heifer | 2 weeks | 500 | Temperature irregular for 4 weeks. The animal grew slowly and was unthrifty for 6 months; otherwise she has remained normal. Still living; age 2 years. |
| 1110 | Heifer | 21 months | 500 | Temperature high on 2nd and 3rd days and on 16th to 24th days after injection. Otherwise she has remained normal. Died from dystocia, aged 2½ years. No indications of tuberculosis found. |
| *A* | Gilt | 3 months | 500 | Irregular temperature between 2nd and 28th day after injection. The day after injection partial paralysis of the rear limbs developed, from which recovery was slow, and the animal was still slightly lame on slaughter 13 weeks after the BCG was injected. Apparently normal throughout on macroscopic inspection except that on close examination in a strong light the lungs were seen to be studded with white nodules up to 0.5 mm in size. A histological study of the lungs showed that they were studded with necrotic foci containing acid-fast bacilli (figs. 17, 18). Guinea pigs inoculated with lung and lymphatic tissue remained normal. |

under the skin. Cold abscesses formed at the points of injection, but the animals have remained in good general condition and are being held under observation.

4. A study has been made of the effects of intravenous injections of BCG into 4 calves in a tuberculosis-free environment. These results are listed in table 15 and serve as a control on the infection experiments described in table 3, as well as showing the reaction to such injections in animals protected from virulent tuberculous infection.

*Discussion of Table 15.*—No permanently injurious effects resulted from the intravenous injection of massive doses of BCG. However, in some animals a disturbance occurred which was evidenced by irregularities in the temperature for a few weeks.

The lung nodules in steer No. 82 and swine *A* consisted of a central mass of acid-fast bacilli, surrounded by a wall of connective tissue. See figures 15–18.

Apparently the lung lesions are resorbed in a few weeks because no trace of lung abnormality was found other than a slight increase in the interlobular connective tissue in animals killed after an interval of more than 4 months.

## HYPERSENSITIVENESS IN VACCINATED CALVES

During the experiments at Berkeley, it has been observed that a rise of temperature may occur in calves soon after revaccination subcutaneously with BCG. The fever begins in from 2 to 6 hours after the injection and ranges between 104° and 106° F for 8 to 14 hours. The temperature curve is similar to the thermal reaction caused by tuberculin and seems to have no relation to the fever which occurs in calves 2 or 3 weeks after the intravenous injection of BCG.

No temperature reactions immediately after the subcutaneous injection of the vaccine have been observed at this Station in tuberculosis-free calves vaccinated for the first time, and we believe that when such reactions do occur, they indicate that the animal has previously been infected by tubercle bacilli or organisms of similar antigenic effect.

Figure 3 shows the temperatures recorded after the vaccination of 3 calves. The records of these animals have been selected for graphic presentation in chart form because they represent three types as explained in the legend under figure 3.

During 1926 and 1927 in accordance with the recommendations of Calmette, the use of tuberculin was avoided when the calves were to

be exposed to virulent infection, although, according to the experiments of Goldenberg of the Ukranian Commission as reported by Tzeknovitzer,[72] such a precaution is probably unnecessary.

Studies of the effect of tuberculin on the vaccinated calves which were protected as much as possible from exposure to virulent bacilli have been made at the California station. In the 20 cases studied, the subcutaneous injection of 100 mg of BCG was always followed by the development of a sensitiveness to tuberculin as evidenced by intradermic and ophthalmic reactions. In the majority of the vaccinated calves, a thermal reaction followed the subcutaneous injection of 500 mg of tuberculin O. T.

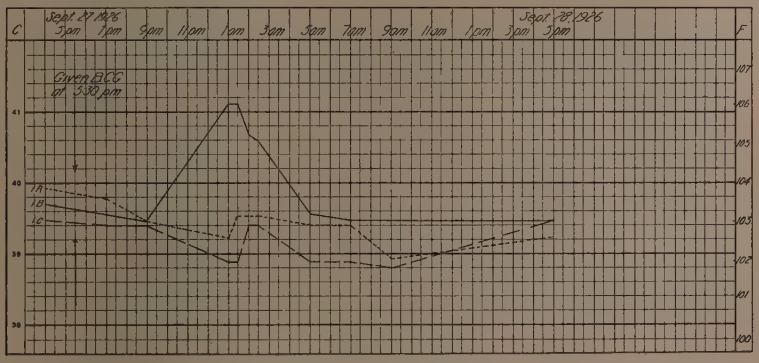


Fig. 3. Temperature chart showing fluctuations in temperature after the injection of BCG into the dewlap.

Calf 1*A* received 40 mg. It had previously received 50 mg on June 6, 1926, and a vaccine abscess had formed, discharged, and healed before September 27, 1926.

Calf 1*B* received 40 mg. It had previously received 50 mg on June 6, 1926. An abscess had formed but did not open. On September 27 it measured 27 x 27 mm.

Calf 1*C* received 50 mg. It had not been vaccinated previously.

The surgical removal of the cold abscess in the dewlap of calf No. 1114 (fig. 8, p. 388) was followed by a gradual reduction in local sensitiveness to tuberculin, although slight traces of sensitiveness to ophthalmic, intradermic, and subcutaneous tuberculin tests persisted for 18 months.

King and Park[40] failed to obtain any indication of increased sensitiveness to tuberculin in guinea pigs and calves that had been fed small doses of BCG.

In order to determine if the feeding of a massive dose of BCG does cause hypersensitiveness, 2 calves at the California station at Berkeley were each given by mouth 1 gram of BCG suspended in physiological

sodium chloride solution. Calf No. 472 was 72 days old at the time the BCG was fed and calf No. 1022 was just born and had never nursed. Sixty days later the calves were each given intradermally 0.2 cc of 100 per cent tuberculin O. T. This provided as strong a dose as is ordinarily used to elicit thermal reaction and at the same time subjected the calves to intradermic test with several times the ordinarily used dose of tuberculin. No thermal reactions were observed. Observations at 60, 84, and 132 hours showed no positive intradermic reactions, although both calves developed slight subcaudal thickenings, the thickenings in calf No. 1022 being slightly the larger ("P" according to the U. S. Bureau of Animal Industry Standard). At the 84th hour observation a sensitizing ophthalmic disc was placed in one eye of each animal and 72 hours later a test dose disk was given each calf. No reactions developed during a 12-hour observation. There was no thermic reaction. Three pre-injection and 10 post-injection temperatures were taken. The latter were started 6½ hours after injection and were taken at 2-hour intervals. Calf No. 472 was butchered at 140 days of age. No indications of tuberculosis were found. Calf No. 1022 is still living and in good condition.

## CONTROL TESTS OF BCG CULTURES ON SMALL ANIMALS

*Guinea Pigs.*—In all, 106 guinea pigs have been used to test the purity and harmlessness of the cultures as required by Calmette. The dosage has ranged from 2 to 100 mg given in various ways. Ninety of these animals have been killed from 2 to 13 months after vaccination. The remainder are being held for observation.

Intramuscular and subcutaneous injections into guinea pigs have usually resulted in the formation of local abscesses which, as a rule, rupture and heal after a few weeks without affecting the general health of the animal. Thirteen months after injection one of the guinea pigs still had about a minim of pus at the point in the thigh where 25 mg had been injected. The pus from these discharging abscesses contained acid-fast bacilli, but at this station subinoculations of such pus into other guinea pigs have thus far always given negative results.

Intraperitoneal injections of 2 to 50 mg into guinea pigs were followed by well-marked adhesions or purulent lesions or both in the abdominal cavity. Such lesions have been demonstrated in all the animals killed 2 to 13 months after inoculation, but the subinoculations thus far have been negative.

*Rabbits.*—Thirteen rabbits have been injected intramuscularly or subcutaneously with doses of BCG ranging from 20 to 100 mg. Local abscesses formed which were usually larger and more persistent than in the guinea pigs. This does not necessarily indicate that rabbits are more susceptible than guinea pigs to specific pathogenicity of BCG. It may be accounted for by the fact that they are apt to develop relatively larger local lesions than guinea pigs when injected intramuscularly or subcutaneously. Besides, the work of Coulaud[29] and others who have injected rather large doses of BCG intravenously into rabbits does not suggest that rabbits are particularly susceptible to BCG, since no generalization of disease is produced; in fact, complete healing has taken place in their cases after several months. One rabbit at the California station which had been injected subcutaneously inside the right thigh with 20 mg was killed after an interval of 105 days. A 30-mm abscess was found between the muscles of the thigh. The liver also contained a white nodule the size of a pea. Acid-fast bacilli were found in smears from both these lesions.

*Swine.*—In an attempt to determine if vaccination has any immunizing effect on swine, injections of BCG have been made into pigs at various ages ranging from 1 day to 6 months old. To date, 45 swine have been vaccinated in various ways, intravenously, subcutaneously, intramuscularly, intradermally, and by mouth. The results are not ready for publication, but as yet no indication has been found that the BCG cultures at this Station are capable of producing generalized or fatal tuberculosis in swine. The lesions are local in character and resemble those produced in calves.

*Comment.*—The fact that the intraperitoneal injection of BCG into guinea pigs and rabbits often causes rather extensive caseous lesions has been the basis of some criticism of the early statements of Calmette as to the harmlessness of BCG. However, it would seem that abdominal lesions resulting from such intraperitoneal injections are of little significance in solving the problem of the possible harmfulness of the subcutaneous and oral vaccination procedures recommended by Calmette.

On the other hand, the cases of generalized tuberculosis in guinea pigs after injection with dissociated BCG cultures reported by Petroff, Branch, and Steenken[61] are of great interest. In their work, however, the technique recommended by Calmette was not followed; instead a deliberate attempt was made to develop virulent strains by dissociating the BCG cultures into R and S types of colonies by growing the bacilli on gentian violet egg media. Chiari, Nobel, and Sole[25] also have reported the cultivation from BCG of a strain virulent for guinea pigs.

Watson[74, 75, 76] was one of the first to question the inoffensiveness of BCG. He reports results from the injection of 134 guinea pigs with BCG as follows:

In 92, or 68.7 per cent, no lesions of tuberculosis were discernible after a duration period averaging 295 days; in 30, or 22.3 per cent, early, slight or localized lesions were found after an average duration of 290 days, and in 12, or 9 per cent, typical generalized tuberculosis developed after an average duration of 309 days.

Watson also questioned the alleged benign character of tuberculous lesions as found in guinea pigs following BCG inoculation and developing in the course of many months. In some cases he reported inoculations made from such so-called benign lesions produced tuberculosis, and, furthermore, serial passage from guinea pig to guinea pig was reported to be productive of typical tuberculosis.

Doyle[31] has claimed that certain parts of Watson's work are open to criticism, but nevertheless, merit immediate repetition in other laboratories.

In experiments at the United States Experiment Station, Bethesda, Maryland, Schroeder and Crawford[65] have reported that in one lot of 36 guinea pigs injected with BCG, 1 died showing lesions of generalized tuberculosis. None of the other 35 were affected. It was not decided whether the 1 guinea pig of this series that died of tuberculosis contracted the disease as a result of the inoculation with BCG or whether this was a case of 'spontaneous' tuberculosis. They stated that when guinea pigs are kept in the same house with tuberculous guinea pigs, there is always a possibility of 'spontaneous' tuberculosis. Sewall[67] has called attention to the occurrence of so-called 'spontaneous' tuberculosis in guinea pigs and the possibility of the introduction of infection to them on comtaminated feed.

The observations of Gerlach,[35] King and Park,[40] Woodruff and Gregory,[79] and many others, as well as the results at the California station, support the contention of Calmette that no increase to high virulence has yet been proved to occur in BCG cultures maintained on ox-bile potato.

At the California Agricultural Experiment Station cultures of BCG have been transplanted on potato in 5 per cent glycerine broth since 1926 and a parallel series has been carried on the same medium with each tenth and eleventh transplant on ox-bile potato containing 5 per cent glycerine. In attempts to dissociate the cultures carried in this way, it was possible at the California station to obtain from the former series variants simulating both R and S types of colonies as described by Petroff, while in the latter series carried according to

Calmette's recommendations, these have not been observed. Guinea pigs inoculated with the S type of colony showed no significant variations from those inoculated with the R type. A slightly enlarged spleen, found in the guinea pig inoculated with the S type, was reinoculated into 2 guinea pigs. These animals showed no evidence of disease when slaughtered at the end of 75 days. One rabbit, inoculated intravenously with about 50 mg of the S type growth, when killed after an interval of 3 months, showed the lungs to be studded with 1 to 2-mm nodules, containing acid-fast bacilli. This lung tissue was reinoculated intramuscularly into 2 guinea pigs. These animals were slaughtered at the end of 75 days and showed no evidence of disease. A rabbit inoculated in a similar way with the R type of growth was in poor condition on autopsy after an interval of 3 months. The lung contained 7 necrotic spots, consisting of creamy pus, 1 to 2 mm in size. The spleen was also slightly enlarged. No acid-fast bacilli or other indications of tuberculosis were demonstrated.

## DISCUSSION AND SUMMARY

Two hundred and eighty-two calves less than 10 days of age, and 15 older cattle were treated with cultures or transplants of cultures received from Calmette. One hundred and ninety-two of the calves were in an extensively tuberculous dairy herd, where vaccinations have been made for 3 years under partially controlled conditions. The remaining 90 calves were from herds free from reactors to the tuberculin test. These have been maintained with an equal number of nonvaccinated controls at the California Agricultural Experiment Station at Berkeley and Davis.

Thirty of the 90 head were used to test the possibility of any injurious effects of BCG on cattle in a presumably tuberculosis-free environment, and the remaining 60 were used to test the immunizing or resistance-producing power of the vaccine by exposing them to tuberculous infection under experimental conditions.

A summary of the results of experimental infection of the 60 head of vaccinated cattle and of 62 of the nonvaccinated controls is given in table 16. This table also contains the results from the infection of 2 calves treated with heat-killed tubercle bacilli and 2 treated with nonvirulent acid-fast bacilli from a culture isolated from bovine lymphangitis.

The results under controlled conditions listed in table 16 indicate that the use of BCG confers upon cattle a definite resistance to tubercle

TABLE 16

SUMMARY FROM PRECEDING TABLES OF RESULTS IN VACCINATED AND CONTROL CALVES

| Number of table in preceding text summarized | Number and sex | Method of vaccination | Form and frequency of infection | Autopsy findings | Conclusions |
|---|---|---|---|---|---|
| 3 | 2 steers | 100 mg BCG in dewlap. | 2 mg bovine tubercle bacilli intravenously. | 1, slaughtered 4½ months after infection, had no lesions, but guinea pigs became infected from the apparently normal lymph-node tissue. The other calf, slaughtered 10 months after infection, had slight but widely distributed lesions. | The BCG subcutaneously gave sufficient resistance to prevent the fatal effects of intravenous infection. |
| 3 | 2 steers | 50 mg BCG intravenously. | 2mg bovine tubercle bacilli intravenously. | 1 died of tuberculous pneumonia 51 days after infection. The other remained apparently normal, but, on slaughter 110 days after infection, was found to have extensive thoracic lesions. | The BCG intravenously apparently retarded fatal results in 1 and prevented fatal effects in the other calf. |
| 4 | 2 steers | 1 gram heat-killed tubercle bacilli intravenously. | 2mg bovine tubercle bacilli intravenously. | Died of miliary tuberculosis in 29 and 31 days, respectively, after infection. | Vaccination with killed tubercle bacilli had no apparent effect. |
| 3 | 4 steers | Controls. | 2 mg bovine tubercle bacilli intravenously. | Controls died of miliary tuberculosis in from 20 to 34 days after infection. | |
| 3 | 2 steers | 100 mg BCG in dewlap. | 50 mg bovine tubercle bacilli injected subcutaneously. | On slaughter after 2½ and 4 months, respectively, both were found to have well-marked lesions at point of infecting injection and slight lesions in certain lymphatic tissues. | An evident resistance was conferred by the vaccine. |
| 3 | 2 steers | Controls. | 50 mg bovine tubercle bacilli injected subcutaneously. | Developed advanced clinical tuberculosis. On slaughter after 2 months, both had generalized tuberculosis. | |

| | | | | | |
|---|---|---|---|---|---|
| 6 | 4 steers | 100 mg BCG in dewlap. | 6 feedings. | 1 calf had extensive mesenteric and slight retropharyngeal lesions; 2 had slight mesenteric lesions and in 1, no lesions were found. | The lesions in the calves vaccinated with BCG were less pronounced than in the respective controls, and the lesions in the calves treated with acid-fast bacilli from a bovine skin lesion culture were more pronounced than in the controls. |
| 6 | 4 steers | Controls. | 6 feedings. | All 4 had extensive caseocalcification of 1 or more groups of lymph nodes. | |
| 6 | 2 steers | 100 mg acid-fast bacilli from a bovine skin lesion. | 2 feedings. | Tuberculous pneumonia from aspiration in 1 and extensive lymphatic caseocalcification in the other. | |
| 6 | 2 steers | Controls. | 2 feedings. | Extensive lymphatic caseocalcification in 1 calf, and retropharyngeal caseation in the other. | |
| 7*A* | 19 steers | 100 mg BCG in dewlap. | 10 feedings. | Of 14 calves infected, 35 or more days after vaccination, 3 had well-marked lesions, 6 had slight lesions, and in 5 no lesions were found. One calf died of gastritis too soon to justify consideration. | In general the lesions in the calves infected 35 days or more after vaccination were less extensive than in the controls. The lesions in 4 calves infected within 25 days after vaccination were widely distributed but not large in size. |
| 7*B* | 19 steers | Controls. | 10 feedings. | 10 had extensive, 4 had well-marked, and 3 had slight lesions. In 1, no lesions were found, but the guinea-pig test was positive. | |
| 8*A* | 10 steers | 100 mg BCG in dewlap. | 30 feedings. | 1 calf had extensive caseation of mesenteric nodes; 1 had well-marked lesions in the spleen and slight lesions in the mesentery; 1 had well-marked mesenteric lesions only; 1 had well-marked mesenteric, cecal, and cervical lesions; and 5 had slight caseous lesions. In 1, no tuberculous lesions were found. | The lesions in the vaccinated calves were less extensive than in their respective controls in 8 animals; in 2, the lesions were about equal to the controls. |
| 8*B* | 10steers | Controls. | 30 feedings. | 5 had well-marked and the other 5 extensive lesions. | |
| 9*A* | 5 steers | 100 mg BCG in dewlap. | 36 feedings. The last 6 were massive. | 4 had slight, but widely distributed, lesions. The other had well-marked cervical and slight mesenteric and ileocecal lymph-node lesions. | The lesions in 4 of the vaccinated calves were less extensive than in their respective controls. In the other vaccinated calf, the lesions were more numerous and widely distributed but smaller in size than in its control. |
| 9*B* | 5 steers | Controls. | 36 feedings. The last 6 were massive. | 3 had extensive and 2 had well-marked lesions. | |

TABLE 16—(*Concluded*)

| Number of table in preceding text summarized | Number and sex | Method of vaccination | Form and frequency of infection | Autopsy findings | Conclusions |
|---|---|---|---|---|---|
| 10*A* | 2 steers | 100 mg BCG in dewlap. | Fed on milk of tuberculous udder, cow No. 600. | 1 had slight mesenteric and well-marked ileocecal lymph-node caseation. The other calf had no demonstrable tuberculous lesions. | The controls were more tuberculous than the vaccinated |
| 10*B* | 2 steers | Controls. | Fed on milk of tuberculous udder, cow No. 600. | Both had well-marked tuberculous lesions. | |
| 10*A* | 2 steers | 100gm BCG in dewlap. | Fed on milk of tuberculous udder, cow No. 2171. | 1 had extensive caseation of ileocecal nodes and well-marked lesions elsewnere; 1 died of intercurrent causes 8 months after infection and the only tuberculous lesion found was a slight retropharyngeal involvement. | Indications point to a protection from the vaccination against a fatal termination of the infection. |
| 10*B* | 2 steers | Controls. | Fed on milk of tuberculous udder, cow No. 2171. | Both died of generalized tuberculosis. | |
| 11*A* | 3 steers | 100 mg BCG intravenously. | Feeding. | 2 had slight abdominal lesions; in the other no lesions were found, but guinea pigs injected with its cervical and mesenteric lymph nodes became tuberculous. | This limited number of cases indicates that the intravenous feeding and intracutaneous methods of vaccination all conferred some resistance to tuberculosis, but there is no evidence that they are superior to the subcutaneous dewlap injection |
| 11*A* | 6 steers | Massive doses of BCG by mouth. | Feeding. | 1 had well-marked tuberculous lesions; 3 slight lesions localized in the retropharyngeal lymph nodes; 1 had widely distributed lesions; and in 1, no lesions were found but guinea pigs injected with its cervical nodes became tuberculous. | |
| 11*A* | 3 steers | 100 mg BCG intracutaneously. | Feeding. | 1 had well-marked and 1 had slight tuberculous lesions. The 3rd died of intercurrent gastritis too soon to be of use. | |
| 11*B* | 12 steers | Controls on above 12 calves. | Feeding. | 10 had extensive tuberculosis and in the other 2 the lesions were well-marked. In all 12 controls the lesions were more pronounced than in any of the corresponding vaccinated calves. | |

bacilli. As already pointed out by Zwick and Witte[80] in a summary of the literature, resistance against artificial infection in this way is of little significance in answering the question of the practical value of the vaccine. This may be true, but natural infection is so beset with variables that definite conclusions from experiments under farm conditions seem unreliable to the writers unless confirmed by controlled experiments under field laboratory conditions. It is hoped that a continuation of the investigations on the cattle in the dairy herd belonging to Mr. H. D. Williamson will add materially to the data necessary for a definite answer.

For purposes of a comparative grouping of the autopsy findings in the 126 animals listed in table 17, a lesion was arbitrarily classed as slight, well-marked, or extensive in accordance with the standard listed in the footnote of table 6. When tabulated under such groupings, the results expressed in table 17 were obtained.

TABLE 17

RELATIVE EXTENT OF THE TUBERCULOUS LESIONS IN CALVES

| Type of lesions | Vaccinated* | Controls | Supplementary controls† |
|---|---|---|---|
| Extensive | 7‡ | 40‡ | 5† |
| Well-marked | 13 | 16 | 1 |
| Slight | 27 | 3 | ..... |
| None visible | 13 | 1 | ..... |
| Total | 60 | 60 | 6 |

* Nine of the calves were subjected to infection within 30 days of vaccination (see tables 7*A*, 9*A*, and 10*A*). If these 9 are eliminated, the tabulated results for vaccinated calves will be changed as follows: Extensive..... 6 Well-marked.....10 Slight.....23 None visible..... 12

† In addition to the 60 controls, use was made of 6 calves as supplementary controls; 2 of these had each been previously treated with an intravenous injection of 1 gram of heat-killed tubercle bacilli; each of 2 others had received a subcutaneous injection of 100 mg of nonvirulent acid-fast bacilli in culture from bovine lymphangitis; and 2 were unvaccinated.

‡ Includes 9 cases of fatal tuberculosis in controls and 2 in vaccinated calves. These 2 animals had been vaccinated in the jugular vein and 4 months later infected through the same channel.

It is evident that the subcutaneous vaccination afforded protection against the fatal effects of intravenous and subcutaneous infection with cultures of virulent bovine tubercle bacilli. On the other hand, the relatively slight differences between the number of lesions in the vaccinated and the control calves which had been subjected to repeated infections through the mouth indicated that no measurable resistance was afforded by the vaccination to the entrance into the tissues of virulent tubercle bacilli, but the tissue damage resulting from such invasion was in most cases less in the vaccinated than in the control calves.

The most marked contrasts between the vaccinated and control calves infected by mouth occurred in the groups described in tables 10*A* and 10*B*, in which the tuberculous infection received by the 4 calves 47, 48, 147, and 148, from the milk of the tuberculous udder of cow No. 2171, was either very virulent or very massive, inasmuch as both controls died of tuberculosis at 62 and 162 days respectively. Vaccinated calf No. 47 remained in good general condition and was butchered 94 days after the first infecting feeding. Widely distributed tuberculous lesions were found, but they were not extensive enough to affect the general condition of the animal. Vaccinated calf No. 48 remained in good general condition until death by accident 246 days after infection. Only slight lesions of tuberculosis were found.

The failure of the subcutaneous vaccination to produce complete immunity to tuberculous feeding infection at the California station led to some preliminary trials on calves at Berkeley in which BCG was introduced into the body through intravenous, intradermic, and oral channels. Tables 11*A* and 11*B* show that these methods also apparently conferred some resistance to feeding infection, but not appreciably greater than that conferred by subcutaneous vaccination. Against the fatal effects of intravenous infection, the subcutaneous method of vaccination was more effective than the intravenous, as judged by the results tabulated in table 3.

A comparison of the autopsy results on the various groups of control calves indicates that the prolonged feeding of massive doses of tubercle bacilli does not usually result in the production of more extensive lesions than when the feeding is stopped at the end of 10 feedings. It may be seen from tables 7*B*, 8*B*, and 9*B* that 10 of the 19 control calves which received 10 feedings had extensive lesions, while only 4 cases of extensive tuberculosis occurred in the 15 control calves which received 30 to 36 feedings. It should be noted that the latter 15 calves had received their first 10 feedings from the same mixtures that were given the first mentioned group of 10 animals.

These results support the generally accepted theory that the lesions resulting from the first tubercle bacilli to invade the body tend to create a resistant power in the tissues against future tuberculous infection. The results, however, are not entirely in accord with the assertions of Calmette[7] that only fresh tuberculous infection, occurring again and again at short intervals, is able to overcome the natural barrier of the mesenteric lymph nodes.

The fact that no macroscopic lesions of tuberculosis could be found in 5 vaccinated heifers (table 14*A*) which were kept exposed to infection for 3 years at the Williamson Ranch, while tuberculous lesions

were found in 5 nonvaccinated heifers of approximately the same age on that ranch tends to support Calmette's claims.

The writers[38] have observed that, under conditions prevailing in certain parts of California, it is not difficult in tuberculous herds to rear heifers free from tuberculosis until they calve for the first time. Owners are then often under the necessity of introducing the disease-free heifers into the infected milking herds. A possible use for BCG may be found in the vaccination of such heifers. In the writers' opinion, if the resistance conferred is sufficient to prevent the development of open lesions, the problem of reducing the incidence of tuberculosis to a point where eradication measures may be economical in such herds will have been solved. In view of this, experiments are being planned at the California station to further test out the safety and practicability of vaccinating tuberculosis-free heifers during their first pregnancy and continuing their revaccination annually after they have been introduced into herds of tuberculous cattle.

## CONCLUSIONS

The subcutaneous vaccination of cattle with 100-mg doses of BCG conferred sufficient resistance to protect against the fatal effects of intravenous or subcutaneous injections of virulent tubercle bacilli.

In feeding trials with virulent tubercle bacilli, the vaccinated cattle showed less extensive lesions, as a rule, than the unvaccinated. The prolongation of the feeding of calves with massive doses of virulent tubercle bacilli apparently had no effect in increasing the number or size of the tuberculous lesions found on autopsy 4 to 12 months later. The calves which received from 2 to 10 infecting feedings had just as extensive lesions, on the whole, as those animals which were fed from 20 to 26 additional doses of much larger numbers of virulent tubercle bacilli. This was observed in both the vaccinated and nonvaccinated groups.

Feeding infection experiments with calves following the intradermic, intravenous, or oral administration of BCG indicated that these methods of vaccination are not superior to the subcutaneous.

The resistance afforded by the vaccine was not sufficient to always prevent the penetration of the walls of the alimentary tract by virulent tubercle bacilli. In most cases this induced a caseation of the cervical and mesenteric lymph nodes. The chief protective effect of BCG seems to be in retarding the extension of tuberculous processes occurring from infection received subsequent to vaccination.

Apparently the subcutaneous method of vaccination has furnished protection against the development of clinical cases of tuberculosis in heifers in a tuberculous dairy herd.

The nonprogressive tuberculous changes or the local vaccination lesions, or both, will render the majority of vaccinated cattle hypersensitive for a time to the intradermal injection of tuberculin, making such animals temporarily unmarketable in California except for beef.

BCG appears to be somewhat effective in protecting against a fatal[10] termination of massive infection.

The resistance to tuberculosis conferred by subcutaneous, intravenous, intradermic, or oral methods of administration of BCG, as used at the California station, is not sufficient to justify the use of the vaccine on cattle where measures designed to eradicate tuberculosis in cattle are being successfully carried out. On the other hand, in countries or localities where control measures are proving ineffective or where eradication seems to be hopeless for many years in the future, the vaccine may eventually be found of economic value to cattle owners by preventing the occurrence of extensive or fatal lesions and by limiting the spread of the disease.

Observations of the effect of BCG in cattle, swine, rabbits, and guinea pigs at the California Agricultural Experiment Station have thus far failed to detect the production of any lesions which could be proved to be virulent by reinoculation.

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[10] The observation that subcutaneous vaccination with BCG protects against the fatal effects of tuberculosis is supported by unpublished results obtained in the routine vaccination of monkeys kept at the George Williams Hooper Foundation for Medical Research, University of California, under the direction of K. F. Meyer. The vaccine used on the monkeys was made at the California Agricultural Experiment Station and was similar to that employed in the cattle experiments.

## LITERATURE[11]


[1] Ascoli, A., E. Gentili, G. Gerosa, A. Mangiarotti, D. Nai, C. Setti, F. Ornodeo Zotini, and A. Bassi.
1927. Expériences de prophylaxie antituberculeuse par le vaccin BCG. Ann. Inst. Pasteur. 41:314–322.

[2] Assis, A. de, and O. Dupont.
1928. Essais de prémunition contre de la tuberculose bovine par le BCG. Ann. Inst. Pasteur. 42:1480–1488.

[3] Baum, Hermann.
1912. Das Lymphgefassystem des Rindes. 170 p. 78 figs. Hirschwald, Berlin.

[4] Breed, R. S., and J. D. Brew.
1916. Counting bacteria by means of the microscope. New York Agr. Exp. Sta. Tech. Bul. 49:1–30.

[5] Brinet, M. P.
1929. Six années de prophylaxie de la tuberculose bovine par le BCG dans une exploitation infectée (1922–1928). Rec. Méd. Vét. d'Alfort. 105:134–136.

[6] Buckley, J. S., and T. Castor.
1928. Regional lymph glands of food animals. U. S. Dept. Agr. Cir. 32:1–30.

[7] Calmette, A.
1907. La tuberculose infantile. Presse Médicale (Paris). 14:833–836.

[8] Calmette, A.
1923. Tubercle bacillus infection and tuberculosis in man and animals. Authorized English translation by W. B. Soper and G. H. Smith. 689 p. Williams-Wilkins Co., Baltimore, Md.

[9] Calmette, A.
1927. Sur la vaccination préventive des enfants nouveau-nés contre la tuberculose par le BCG. Ann. Inst. Pasteur. 41:201–232.

[10] Calmette, A.
1927. Technic des cultures de BCG. Ann. Inst. Pasteur. 41:358–365.

[11] Calmette, A.
1928. La prémuntion ou vaccination preventive des nouveau-nés contre la tuberculose par le BCG. Ann. Inst. Pasteur. 42:1–34.

[12] Calmette, A.
1928. L'infection bacillaire et la tuberculose chez l'homme et chez les animaux. 3d ed. 771–798. Masson et Cie., Paris.

[13] Calmette, A., A. Boquet, L. Négre, et C. Guérin.
1926. Prémunition des nouveau-nés contre la tuberculose par le vaccine BCG (1921 a 1926). Ann. Inst. Pasteur. 40:89–133.


[11] The list of literature contains references not discussed in the text. These are included because they relate to work with BCG on cattle.

[14] Calmette, A., and C. Guérin.
1908. Nouvelle contribution à l'étude de la vaccination des bovidés contre la tuberculose. Ann. Inst. Pasteur. 22:689–703.

[15] Calmette, A., and C. Guérin.
1911. Recherches expérimentales sur la defense de l'organisme contre l'infection tuberculeuse. Ann. Inst. Pasteur. 25:625–641.

[16] Calmette, A., and C. Guérin.
1913. Nouvelles recherches expérimentales sur la vaccination des bovidés contre la tuberculose et sur le sort des bouilles tuberculeux dans l'organisme des vaccines. Ann. Inst. Pasteur. 27:162–169.

[17] Calmette, A., and C. Guérin.
1913. Vaccination des bovidés contre la tuberculose. Ann. Inst. Pasteur. 27:162–169. 28:329–337.

[18] Calmette, A., and C. Guérin.
1914. Contribution à l'étude de l'immunite antituberculeuse chez bovidés. Ann. Inst. Pasteur. 28:329–337.

[19] Calmette, A., and C. Guérin.
1920. Nouvelles recherches experimentales sur la vaccination des bovidés contre la tuberculose. Ann. Inst. Pasteur. 34:553–560.

[20] Calmette, A., and C. Guérin.
1924. Vaccination des bovidés contre la tuberculose et methode nouvelle de prophylaxie de la tuberculose bovine. Ann. Inst. Pasteur. 38:371–398.

[21] Calmette, A., and C. Guérin.
1928. Sur le stade lymphatique de l'infection tuberculeuse chez les bovidés. Ann. Inst. Pasteur. 42:175–178.

[22] Calmette, A., C. Guérin, and M. Breton.
1907. Tuberculose expérimentale du cobaye. Ann. Inst. Pasteur. 21:401–416.

[23] Calmette, A., C. Guérin, L. Nègre, and A. Boquet.
1926. Note sur le controle du BCG par l'expérimentation sur le lapin et sur le cobaye. Ann. Inst. Pasteur. 40:574–581.

[24] Calmette, A. and H. Plotz.
1929. Protective inoculation against tuberculosis with BCG. Amer. Rev. Tuber. 19:567–572.

[25] Chiari, H., E. Nobel, and A. Solé.
1928. Versuche mit dem BCG Stamm Calmette. Zeitschr. f. Tub. 50:24–36.

[26] Cobbett, L.
1917. The causes of tuberculosis. 686 p. Cambridge Univ. Press. London.

[27] Conférence Internationale du BCG.
1928. 15–20 Octobre. Rec. de Méd. Vét. 104:691–694.

[28] Conférence Internationale du BCG.
1928. Section d'Hygiene de la Societé des Nations. Ann. Inst. Pasteur, Supplément, 42:59–60.

[29] Coulaud, E.
1927. Effets des injections intraveineuses massives de bacilli bilié (BCG). Ann. Inst. Pasteur. 41:289–296.

[30] Crawford, A. B.
1927. A sensitization method of differentiating avian from mammalian tubercle bacilli. Amer. Rev. Tuberc. **15**:111–118.

[31] Doyle, T. M.
1929. Immunization of bovines against tuberculosis with BCG vaccine. Vet. Record. **9**:209–211.

[32] Dwykoff, P. P., and L. P. Masourowski.
1927. Sur la vaccination par le BCG. Ann. Inst. Pasteur. **41**:1194–1197.

[33] Fénelon, R.
1928. Trois anneés de prophylaxie de la tuberculose bovine par la vaccin BCG dans deux exploitations rurales. Rec. de Méd. Vét., Alfort, **104**:264–265.

[34] Gerlach, F.
1927. Zur Frage der Schutzimpfung gegen Tuberkulose nach Calmette. Centbl. Bakt. Orig., I, **104**:61–65.

[35] Gerlach, F.
1929. Nouvelles recherches sur le bacille tuberculeux BCG. Rev. Gén. de Méd. Vét. **38**:392–399.

[36] Guérin, C.
1928. Prophylaxie gegen Tuberkulose-Infektionen bei Rindern mittels BCG. Wien klin. Wochnschr., **41**:731–735.

[37] Guérin, C., A. Richard, and M. Boissiere.
1927. Essai de prophylaxie de la tuberculose bovine par le BCG dans une exploitation rurale infectée (1921–1927). Ann. Inst. Pasteur. **41**:233–253.

[38] Haring, C. M., and J. Traum.
1928. Bovine tuberculosis. California Agr. Ext. Cir. **21**:1–24.

[39] Harnach, R.
1928. Essais de vaccination des oiseaux contre l'infection tuberculeuse par le BCG du type aviare. Ann. Inst. Pasteur. **42**:382–393.

[40] King, M. J. and W. H. Park.
1929. Effect of Calmette's BCG vaccine on experimental animals. Amer. Jour. Pub. Health. **19**:179–192.

[41] Kraus, R.
1927. Zur Frage der präventiven Schutzimpfung gegen Tuberkulose nach Calmette mittels BCG. Wien. klin. Wochnschr. **40**:49–54.

[42] Kraus, R.
1927. Zulässigkeit der präventiven Schutzimpfung gegen Tuberkulose nach Calmette. Wien. Klin. Wochnschr. **40**:1508–1510.

[43] Kraus, R.
1928. Über die Grundlagen der Schutzimpfung gegen Tuberkulose nach Calmette mit BCG. Kolle-Wasserman Handbuch Path. Mikroorganismen. **5**:887–917.

[44] Lange, B., and K. Lydtin.
1928. Experimentelle Untersuchungen am Rindern über die Schutzwirkung der Kultur BCG (Calmette). Zeitschr. Hygiene u. Infekt. **108**:808–819.

[45] Lange, B., and K. Lydtin.
1928. Experimentelle Untersuchungen an Meerschweinschen und Kaninchen über die Schutzwerkung der Kultur BCG (Calmette). Zeitschr. f. Tuberkl. 50:45–60.

[46] Larson, W. P., and W. A. Evans.
1929. A two-year experiment with the "Calmette" method of vaccination. Jour. Amer. Vet. Med. Assn. 74:58–585.

[47] Lignieres, J.
1928. La police sanitaire de la tuberculose bovine. Quelques considerations sur les consequences de la prémunition par le BCG. Bul. Acad. Vétérinaire. 1:269–272.

[48] Lignieres, J.
1927. Contribution a l'étude des qualites pathogenes du vaccin BCG contre la tuberculose. Bul. de l'Acad. de Méd. [Paris] 98:127–145.

[49] Lignieres, J.
1928. Discussion before the French Academy of Medicine on the value of the BCG vaccine. Jour. Amer. Med. Assoc. 90:1884.

[50] Lowenstein, E.
1928. Tuberkuloseimmunitat. Kolle-Wasserman Handbuch Path. Mikroorganismen. 5:777–886.

[51] Mello, N.
1927. Immunization antituberculeuse des bovidés par le vaccin BCG Annales de la Station Experimentale pour les Maladies Infectieuses du Bétail; Turin. Abstract in Rev. Gén. Méd. Vét. 38:212, 1929.

[52] Metalnikov, S., and V. Secreteva.
1927. Phagocytose et destruction des bacilles tuberculeux. Ann. Inst. Pasteur. 41:301–313.

[53] Mouquet, A.
1928. Note sur l'emploi de vaccin antituberculeuse BCG à la Ménagerie du Museum National d'Histoire Naturelle de Paris. Ann. Inst. Pasteur Supplement. 42:101–109.

[54] Nobel, E.
1928. Tuberkuloseimmunität und Schutzimpfung nach Calmette mit BCG. Wien. klin. Wchnschr. 41:798–800.

[55] Nobel, E.
1928. Tuberkuloseprophylaxe und Calmettesche Schutzimpfung gegen Tuberkulose. Wien. Med. Wochnschr. 78:739.

[56] Oboukhovski.
1928. Suite des expériences de vaccination des bovidés. [Experiments reported by M. Tzeknovitzer in Documents de la Commission Ukrainienne; Troisième rapport.] Ann. Inst. Pasteur. 42:72.

[57] Park, W. H.
1927. Survey of broad phases of tuberculosis. Amer. Rev. Tuberc. 15: 201–220.

[58] Petroff, S. A.
1927. Immunity in tuberculosis. Jour. Amer. Med. Assn. 89:285–293.

[59] Petroff, S. A.
1929. New analysis of value and safety of protective immunization with BCG (Bacillus Calmette-Guérin). Amer. Rev. Tuber. **20**:275–296.

[60] Petroff, S. A., A. Branch, and F. B. Jennings.
1929. Immunological studies in tuberculosis. V. Resistance of animals sensitized with heat-killed tubercle bacilli to measured infecting doses. Jour. Immunol. **16**:233–257.

[61] Petroff, S. A., A. Branch, and W. Steenken, Jr.
1927. Microbic dissociation. III. BCG. Proc. Soc. Exp. Biol. and Med. **25**:14–16.

[62] Petroff, S. A., A. Branch, and W. Steenken, Jr.
1929. A study of Bacillus Calmette-Guérin (BCG). Amer. Rev. Tuberc. **19**:9–46.

[63] Rankin, A. C.
1929. Vaccination against tuberculosis with Bacillus Calmette-Guérin. Canadian Jour. Res. **1**:48–85.

[64] Sanz, B.
1926. Informe del Director del Instituto Biológico. Memoria. **6**:31–32.

[65] Schroeder, E. C., and A. B. Crawford.
1929. Studies concerning the Calmette-Guérin method of vaccinating animals against tuberculosis. Jour. Amer. Vet. Med. Assn. **74**:733–782.

[66] Schrötter, H.
1927. Gesichtspunkte zum Schutzverfahren der Sauglinge gegen Tuberkulose nach A. Calmette. Wein. klin. Wochnschr. **40**:151–154, 194–195. Abs. in Centb. f. Bakt. **87**:274. 1927.

[67] Sewall, H.
1928. A possible source of so-called spontaneous tuberculosis in guinea pigs. Amer. Rev. Tuberc. **18**:829–831.

[68] Suarez, E.
1927. Uber Schutzimpfung gegen Tuberkulose mit BCG nach Calmette. Wien. klin. Wochnschr. **50**:381–382.

[69] Tzeknovitzer, M.
1926. Sur le vaccination antituberculeuse par le BCG. Ann. Inst. Pasteur. **40**:827–829.

[70] Tzeknovitzer, M.
1927. Étude de la vaccination antituberculose par le BCG. Ann. Inst. Pasteur. **41**:322–357.

[71] Tzeknovitzer, M.
1928. Nouvelles expériences sur le vaccin antituberculeux BCG. Ann. Inst. Pasteur. **42**:246–255.

[72] Tzeknovitzer, M.
1928. Documents de la Commission Ukrainienne; Troisième rapport. Ann. Inst. Pasteur, Sup. **42**:67–76.

[73] Wallgren, A.
1928. Intradermal vaccinations with BCG virus. Jour. Amer. Med. Assoc. **91**:1876–1880.

[74] WATSON, E. A.
1927. Tuberculosis research. Jour. Amer. Vet. Med. Assoc. **71**:732–741.

[75] WATSON, E. A.
1928. Tuberculosis control and research. Vet. Record. **8**:394–398.

[76] WATSON, E. A.
1929. Tuberculosis and research problems with particular reference to BCG vaccination. Report Veterinary Director General, Canada. Appendix. **1**:40–47.

[77] WATSON, E. A., C. W. MCINTOSH, and H. KONST.
1928. Research on Bacillus Calmette-Guérin and experimental vaccination against bovine tuberculosis. Jour. Amer. Vet. Med. Assoc. **73**:799–816.

[78] WEBB, G. B.
1929. Immunization against tuberculosis. Jour. Amer. Med. Assn. **93**:1459–1461.

[79] WOODRUFF, H. A., and T. S. GREGORY.
1928. The prevention of tuberculosis in cattle. An investigation to determine the value of the BCG vaccine for the prevention of tuberculosis. Jour. Council Scientific and Industrial Research [Australia]. **1**:158–162.

[80] ZWICK, W., and J. WITTE.
1928. Schutzimpfung mit durch Galle abgeschwächten Tuberkelbazillen (Calmette-Guérin). Kolle-Wasserman Handbuch Path. Mikroorganismen. **5**:1025–1029.

FIGURES 4–18

Fig. 4

Fig. 5

Figs. 4 and 5. Appearance of the tuberculous lesions in the necks of vaccinated calves 5 and 6, 60 days after the subcutaneous injection of 50 mg of virulent tubercle bacilli into the left side of the neck. Compare with figures 6 and 7.

Fig. 6

Fig. 7

Figs. 6 and 7. Nonvaccinated control calves 105 and 106, showing appearance 60 days after the injection of 50 mg of tubercle bacilli.

Fig. 8. Abscess in the dewlap (calf No. 1114) typical of the usual effect from the injection of 100 mg of BCG. Eight months after injection, on the day the above photograph was taken, the abscess and its surrounding tissues were removed surgically. Intradermic hypersensitiveness to tuberculin gradually decreased in this calf, although after 18 months the animal was found to be still slightly hypersensitive to ophthalmic and intradermic applications of tuberculin.

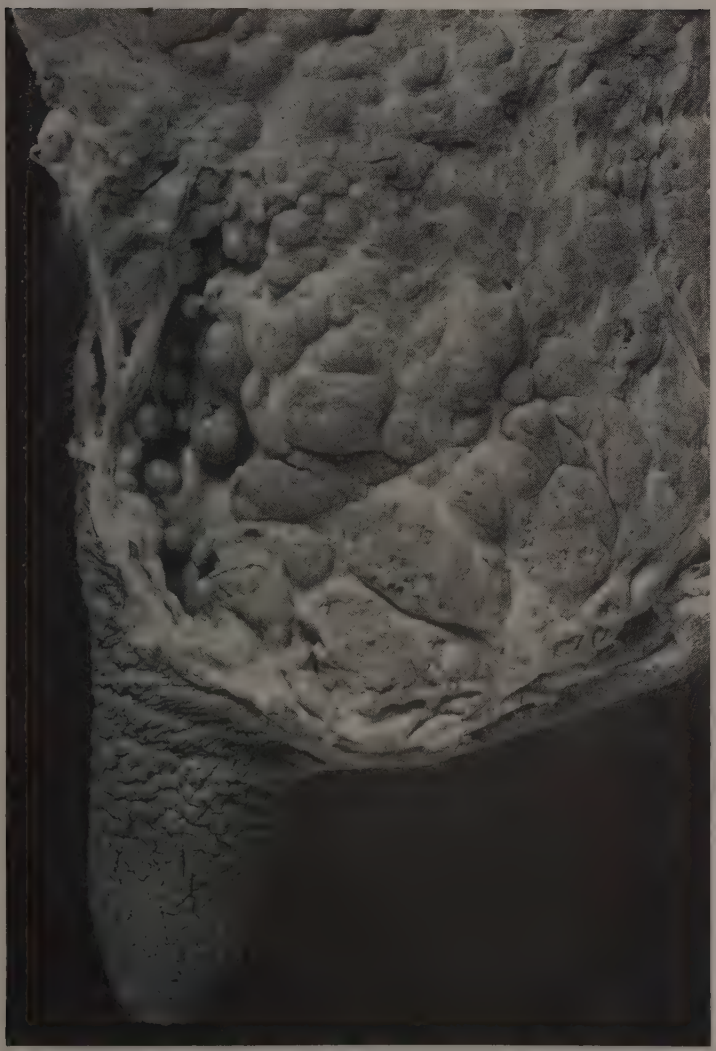

Fig. 9. Section of the left fore quarter of the udder of cow No. 600, showing extensive tuberculous lesions. The right rear quarter of this cow was similarly affected. The milk from this cow was used for infection feedings on vaccinated calves 45 and 46 and nonvaccinated control calves 145 and 146, and resulted in moderate tuberculous lesions in all four. See tables 10*A* and 10*B*.

Fig. 10. Appearance of the udder of cow No. 2171 on November 23, 1927. Tuberculous induration had distended the right rear quarter so that the teat of that quarter was shortened and displaced toward the left and to the front. The milk from this cow was used for infection feedings on vaccinated calves 47 and 48 and nonvaccinated control calves 147 and 148, and resulted in well-marked or extensive infection in all four. The vaccinated calves remained in good general condition. The nonvaccinated controls died of tuberculosis. See tables 10*A* and 10*B*.

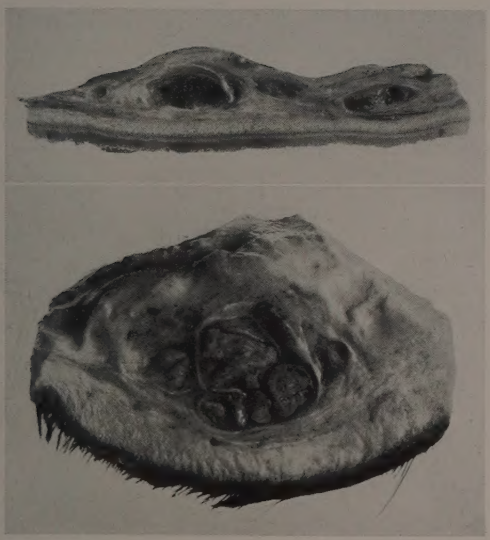

Fig. 11. Typical lesions caused by the injection of 100 mg BCG into the dewlaps of calves. Cross sections actual size. Upper from calf No. 38, removed at autopsy 246 days after vaccination (see table 8*A*). Lower from calf No. 31, removed 447 days after vaccination (see table 8*A*). Acid-fast bacilli were abundant in smears from these lesions.

See page 393 for illustrations.

Fig. 12. Transections of mesenteric and ileocecal lymph-nodes, showing caseous and caseocalcareous areas. The normal tissues have been partially trimed away. This illustration shows in natural size all of the tuberculous lesions found in vaccinated calf No. 38. Compare with lesion symbols for this calf in table 8*A*.

Fig. 13. Transections of caseous and caseocalcareous lymph nodes from control calf No. 138. Left to right, top row, left retropharyngeal, right prescapular, right retropharyngeal; second row, anterior and median mediastinal and three mesenteric; third row, five mesenteric; lowest row, two mesenteric, one colic, one gastrohepatic. The normal tissue has been partially trimmed away from each node shown. No lesions were found in calf No. 138 other than those shown in figures 13 and 14, except in the retropharyngeals which were studded throughout as shown on the cut surfaces in figure 13. (Natural size.)

Fig. 14. Appearance, natural size, of a caseous nodule in a Peyer's patch, ileum of calf No. 138. The mucous membrane has been removed to show the caseous material beneath. Acid-fast bacilli were demonstrated to be present in large numbers and inoculated guinea pigs developed tuberculosis.

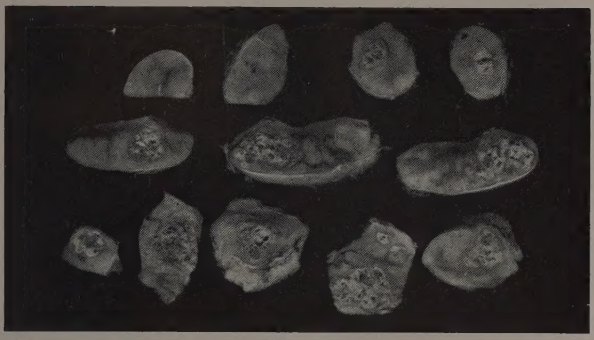

Fig. 12

Fig. 13

Fig. 14

See page 392 for explanation of above.

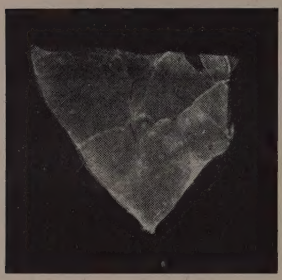

Fig. 15

Fig. 16

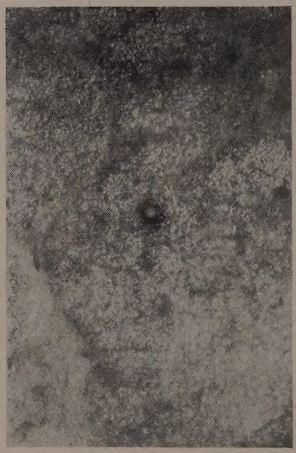

Fig. 17

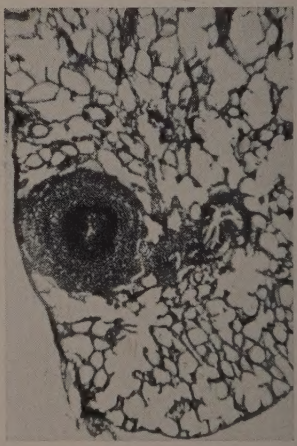

Fig. 18

Fig. 15. Appearance of the surface of a portion of the lung, natural size, pleura intact, of calf No. 82 (see table 15). The white specks are lesions resulting from the intravenous injection of BCG. Calf No. 82 was butchered while in apparently good health 30 days after the injection of 100 mg of BCG into a jugular vein.

Fig. 16. Appearance of a section through one of the nodules in the lung of calf No. 82. The black mass in the center consisted of clumped acid-fast bacilli. Staining: Ziehl-Neelsen and methylene blue. (Enlarged 20 x.)

Fig. 17. Appearance of the surface of the lung, pleura intact, of swine *A*, showing a typical nodule caused by BCG (see table 15). (Enlarged 5 x.)

Fig. 18. Appearance of a section through one of the nodules in the lung of swine *A*. The dark mass in the center consisted chiefly of acid-fast bacilli. Staining: Ziehl-Neelsen and methylene blue. (Enlarged 40 x.)